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14. ABSTRACT
This project is focused on novel tumor vaccines directed at MUC1 and other tumor antigens. Our specific aims are: 1)To assess the effectiveness of vaccines against MUC1 and other tumor antigens in the prevention and treatment of spontaneous breast carcinomas in mice; 2)To translate an effective vaccine strategy into a phase I clinical trial in patients with undetectable disease following standard therapy. The model of spontaneous mammary cancer is the MUC1-expressing polyoma middle T antigen mice (MMT). We have tested five vaccines in the preclinical mouse model and all elicited a strong immune response. The vaccine using MUC1 class I binding peptides prevented MUC1-expressing tumor growth. We have designed the Phase I clinical trial using a peptide vaccine comprised of MUC1 and HER-2/neu MHC class I peptides and HER-2/neu MHC class II peptide with unmethylated CpG oligodeoxynucleotides and GM-CSF as adjuvants in breast cancer patients free of disease. The clinical trial was unanimously approved by the Mayo Institutional Review Board (IRB 582-05) following receipt of FDA approval (BB-IND 12155) and by the DoD HSRRB in January 2007. Following receipt of the approvals, Pfizer agreed to supply the CpG7909(PF-3512676) for the clinical trial, as Pfizer has licensed the CpG from Coley Pharmaceuticals. Amended documents showing the change in supplier of CpG were submitted to the DoD HSRRB for final approval and to the FDA. Final approval from the DoD HRPO was received June 9, 2008. The clinical trial opened August 28, 2008. Thirty-one patients have been enrolled.

15. SUBJECT TERMS
Immunotherapy, vaccine, MUC1, mucin, mouse model, tolerance

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INTRODUCTION

This project is focused on the development of novel tumor vaccines directed at MUC1, a transmembrane mucin that is aberrantly expressed in cancer. MUC1 is expressed on greater than 90% of breast cancers and often elicits cellular and humoral immune responses in humans. However, these responses are not sufficiently strong to eradicate tumors. MUC1 is a candidate peptide for novel immunotherapy strategies to strongly activate the immune system to eradicate tumors expressing these epitopes. In tumors, there is strong over expression of MUC1 on tumor cells and in circulation, expression is no longer restricted to the apical domain of cells, and glycosylation is altered, revealing immunodominant tumor-specific peptide sequences.

In our preclinical studies we have utilized mice that develop spontaneous mammary gland cancer that express MUC1. MUC1 transgenic mice (MUC1.Tg) were bred with mice carrying the MMTV-driven polyoma middle T antigen (MT) to create MMT mice. Mice transgenic for this protein develop B and T cell tolerance and are refractory to immunization with the protein encoded by the transgene. All mice are congenic on the C57BL/6 background to eliminate strain-specific modifier effects. In the MMT mice, mammary gland tumors are induced by the action of a potent tyrosine kinase activity associated with the polyoma virus middle T antigen driven by the mouse mammary tumor virus long terminal repeat (MMTV) [2]. Middle T specifically associates with and activates the tyrosine kinase activity of a number of c-src family members, eliciting tumors when a threshold level of gene product has been attained. This promoter is transcriptionally active throughout all stages of mammary gland development and results in widespread transformation of the mammary epithelium and the rapid production of multifocal mammary adenocarcinomas in 100% of the female mice. The MMT mouse appears to be an appropriate model for human cancer and allows us to study the effects of self-tolerance, immunity and auto-immunity to MUC1 as mammary tumors develop spontaneously.

The **hypothesis** of our study is that enhancing MUC1-specific immunity will result in anti-tumor immunity. We propose to develop an optimal cancer vaccine using epithelial cell mucin MUC1 peptides or protein or MUC1-expressing tumors presented by DCs as immunogen. The most successful therapies will be tested in a phase I clinical trial. An additional hypothesis is that tolerance occurs within the tumor environment, although immunization strategies can be developed to overcome tolerance.

RESULTS (BODY)

Specific Aims:

Specific Aim 1: To assess the effectiveness of vaccine formulations against MUC1 and other tumor antigens in the prevention and treatment of spontaneous breast carcinomas in mice.

Our preclinical studies were completed at the end of year three and the papers describing the results were included in the progress report for 2006.

Specific Aim 2: To translate the most effective vaccine strategies into phase I clinical trials in patients with high and low tumor burden.

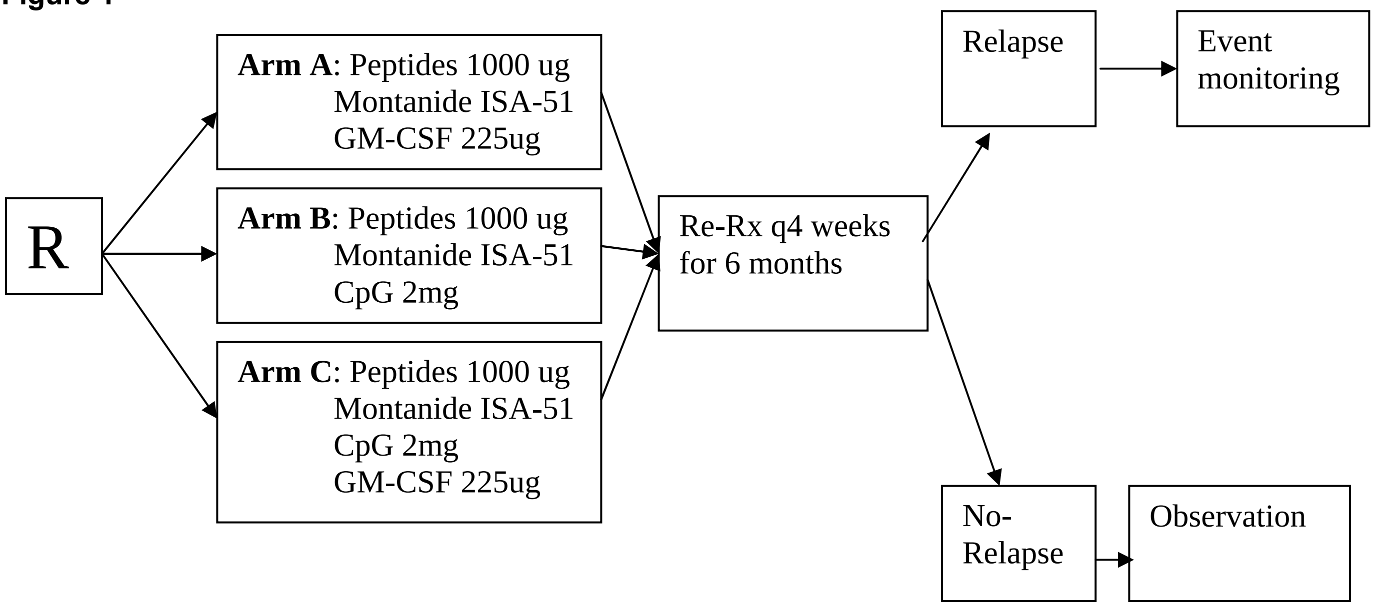
Section II - Description of Regulatory Status of the Clinical Trial

The clinical trial opened August 28, 2008, at all three Mayo Clinic sites. On October 26, 2009, 31 patients have been registered out of the anticipated 45 patients. A number of patients are in pre-registration. Pre-registration involves determination of HLA-A2 status (must be positive) and MUC1 positivity on immunohistochemistry of invasive carcinoma. Following these analyses, the patient undergoes a physical exam and blood work.

The trial is testing the MUC1 class I peptide (STAPPVHNV), HER-2/neu class I peptide (ILHNGAYSL) and HER-2/neu class II peptide (KVPIKWMALESIL) (1000 µg of each peptide) delivered in Montanide ISA-51 and compare GM-CSF with unmethylated CpG oligodeoxynucleotides (PF 3512676) as immune adjuvants. Few vaccines have been tested in the optimal setting of minimal residual disease. CpG unmethylated oligodeoxynucleotides are a novel adjuvant that promote strong, antigen-specific T cell responses and help to overcome immune tolerance.

The schema for the clinical trial is shown (Fig. 1).

Figure 1



Recruitment Plan and No-Cost Extension

The protocol MCO338 entitled "MUC1/HER-2/neu Peptide Based Immunotherapeutic Vaccines for Breast Adenocarcinomas" opened on August 28, 2008. The trial was closed briefly from 2/24/09 until 5/19/09 due to toxicity. The dose of CpG ODN (PFT PF 3512676) was reduced to 1 mg (from 2 mg) in arms B and C and there have been no additional reports of toxicity. Thus, the trial has been open for approximately 10 months. In that time we have accrued 31 patients by October 28, 2009. This number included 15 patients from Mayo Clinic Arizona, 14 patients from Mayo Clinic Rochester, and 2 patients from Mayo Clinic Florida. There is a projected enrollment of 45 patients. In ten months 69% of our projected enrollment has been achieved. This averages to 3 patients per month, which would mean that we would reach our projected enrollment in about 5 months. Since the described trial includes a two-year follow-up period, with blood draws and immune monitoring occurring until 2 years after the first vaccination, we requested a three-year no-cost extension until August 14, 2012 would enable us to complete enrollment and follow-up and analyses. We anticipate completing enrollment in March, 2010; two-year follow-up would end in March 2012. A completion date of August 14, 2012 was granted on 10/26/09 to allow us to complete the studies and write the papers and final report. The assistance agreement is attached to this report.

KEY RESEARCH ACCOMPLISHMENTS

- The preclinical research was completed and described in the annual reports for years 3 and 4.

REPORTABLE OUTCOMES

- The Clinical Trial was activated on August 28, 2008, at Mayo Clinic Arizona, Mayo Clinic Rochester, Minnesota and Mayo Clinic Jacksonville, Florida. The activation memo is included in the Appendix.

- The clinical trial has enrolled 31 of the 45 anticipated patients (69%).

Time Table of Protocol Development

- Clinical protocol concept approved by Mayo Clinic Cancer Center 12-11-03
- Completed Mayo Clinic Cancer Center Peer Review process 5-4-04
- List of recommendations by FDA (pre IND conference) 4-21-04
- Peptides synthesized and vialed by ClinAlfa® for use in this clinical trial:
  1. Her-2/neu (435-443)
  2. Her-2/neu (883-899)
  3. MUC1 (950-958)
- Completion of IND documentation and submission to FDA on December 17, 2004.
- FDA approval (IND # 12155)
- Mayo IRB approval April 22, 2005 (IRB 782-05)
- Submission to DOD HSRRB on May 11, 2005
- Submission to FDA of the revised 1572 and Investigator's Brochure on September 15, 2005
- Submission to Mayo IRB of amendment, which excludes prisoners from the study population and reduces the number of personnel involved in the study (September 12, 2005)
- Submission of revision to HSRRB on February 10, 2006 (response to request for revisions from 14 December 2005 HSRRB meeting)
- Submission of revision to HSRRB on May 18, 2006
- Submission of final documents to the Mayo IRB August 30, 2006
- Final approval Mayo IRB December 15, 2006
- Final approval DoD HSRRB (Log Number A-10856) January 26, 2007
- CpG-7909 adjuvant to be supplied by Pfizer as PF3512676 for this clinical trial.
- Submission of revised clinical protocol to Mayo IRB, HSRRB and IND (July, August 2007)
- Final Mayo IRB approval August 2, 2007
- Extension of "performance period" by 24 months to 14 September 2009
- Approval from DOD HRPO on June 9, 2008
- Clinical Trial was activated August 28, 2008.
- Clinical Trial was closed from 2/24/09 to 5/19/09 due to toxicitiy at injection sites. The amount of CpG ODN adjuvant was reduced from 2 mg to 1 mg and the trial was reopened. There have been no further reports of toxicities.
- Enrollment is 69% completed.

CONCLUSIONS

The trial opened August 28, 2008 and to date it has accrued 31 of the anticipated 45 patients. Everything is proceeding well.

APPENDICES

The appendix includes the activation notification and other MC0339 clinical trial documents.

A – Assistance Agreement

B – MC0338 clinical trial protocol “MUC1/HER-2/neu Peptide Based Immunotherapeutic Vaccines for Breast Adenocarcinomas” Modified 5-19-09

C – Consent From

D – Revised SOW (submitted 5-15-09)

ASSISTANCE AGREEMENT

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PROJECT TITLE: "Immunotherapeutic Strategies in Breast Cancer: Preclinical and Clinical Trials"

CFDA 12.420

PERFORMANCE PERIOD: 15 Aug 2001 - 14 Sep 2012 (Research ends 14 August 2012)	PRINCIPAL INVESTIGATOR: Sandra J. Gendler, Ph.D
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AWARDED TO: Mayo Clinic Scottsdale 13400 E. Shea Blvd. Scottsdale, AZ 85259	REMIT PAYMENT TO: Mayo Clinic Scottsdale 13400 E. Shea Blvd. Scottsdale, AZ 85259

ACCOUNTING AND APPROPRIATION DATA:
Not applicable

SCOPE OF WORK:

Breast Cancer Research Program Clinical Translation Award

In accordance with the Mutual agreement of the parties, and the recipient's request dated 9 October 2009, which is incorporated herein by reference, the Assistance Agreement Period of Performance is extended by 36 months at no additional cost. This change includes a revised SOW dated 15 May 2009, incorporated herein by reference.

FROM: 15 August 2001 - 14 September 2009 (Research to be completed 14 August 2009)

TO: 15 August 2001 - 14 September 2012 (Research to be completed 14 August 2012)

All other terms and conditions remain unchanged.

Award Total Amount: $1,901,322.00

RECIPIENT		GRANTS OFFICER	
ACCEPTED BY: No signature required. Reference recipient's request dated 9 Oct 2009. SIGNATURE		UNITED STATES OF AMERICA Pamela L. Fisher SIGNATURE	
NAME AND TITLE	DATE	NAME AND TITLE Pamela L. Fisher GRANTS OFFICER	DATE 10/26/2009

USAMRAA FORM 60-R, Feb 00

Mayo Clinic Cancer Center

MUC1/HER-2/neu Peptide Based Immunotherapeutic Vaccines for Breast Adenocarcinomas

Principal Investigators/Study Chairs:	Svetomir Markovic, M.D., Ph.D. *+ Mayo Clinic Cancer Center 200 First Street, SW Rochester, MN 55905 507/284–2511 507/284-5280 (FAX)
	Sandra J. Gendler, Ph.D. √ Mayo Clinic Scottsdale 13400 E. Shea Boulevard Scottsdale, AZ 85255 480/301-7062 480/301-7017 (FAX)
Study Co-chairs:	James N. Ingle, M.D (Mayo Clinic, Rochester)
	Tom Fitch, M.D (Mayo Clinic, Scottsdale) Barbara Pockaj, M.D (Mayo Clinic, Scottsdale) Edith A. Perez, M.D. (Mayo Clinic, Jacksonville)
Statistician:	Vera J. Suman, Ph.D. √

* Investigator having primary responsibility for this protocol
\+ IND sponsor (IND# 12155)
√ Study contributor(s) not responsible for patient care.

Document History	**(effective date)**
Activation	August 28, 2008
MCCC Addendum 1	May 19, 2009

Protocol Resources

Questions:	Contact Name:
Review all unanticipated problems involving risk to volunteers or others, serious adverse events and all volunteer deaths associated with the protocol and provide an unbiased written report of the event	Robert R. McWilliams, M.D. Medical Monitor Phone: 507-284-8432 E-mail: mcwilliams.robert@mayo.edu
Patient eligibility*****, test schedule, treatment delays/interruptions/adjustments, dose modifications, adverse events, forms completion	Carol Leonard Quality Control Specialist Phone: 507-284-3121 Fax: 507-284-1902 E-mail: leonard@mayo.edu
Drug administration, infusion pumps, nursing guidelines	Lisa Kottschade, NP Mayo Clinic Cancer Center Nurse Phone: 507-538-2958 E-mail: kottschade.lisa@mayo.edu
Clinical data submission and record maintenance	Renee Bradshaw Clinic Research Associate Phone: 507-284-2041 E-mail: Bradshaw.renee@mayo.edu
Protocol document, consent form, Regulatory issues	Jane M. Milburn, MBA Protocol Development Coordinator Phone: 507-266-0743 Fax: 507-284-5280 E-mail: milburn@mayo.edu
Technical problems with electronic form entry	Vicki Bryhn Data Management Specialist Phone: 507-266-5350 Fax:: 507-538-0906 E-mail: bryhn@mayo.edu

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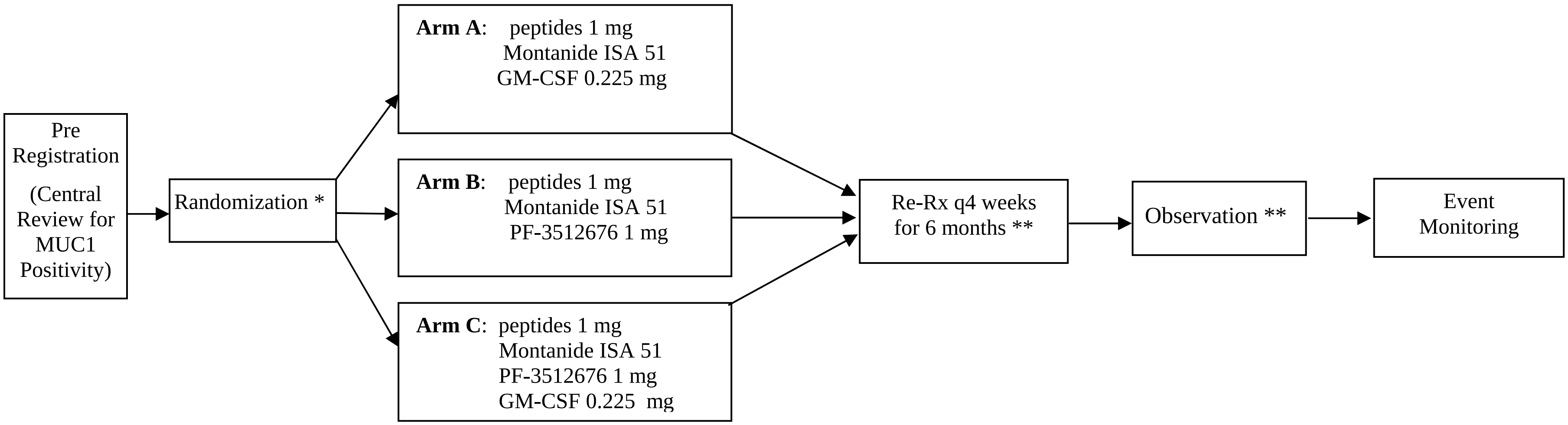
Schema



* Patient must have MUC1 positive breast cancer confirmed by central review prior to randomization.
** At relapse, patients enter event monitoring phase (see Section 13)

Generic Name	Brand Name	Mayo Abbreviation	Availability
MUC1 (STAPPVHNV)		MUC1	Clinalfa
HER2 Peptide-1 (ILHNGAYSL)		HER2-1	Clinalfa
HER2 Peptide 2 (KVPIKWMALESILRRRF)		HER2-2	Clinalfa
Montanide ISA-51 or Montanide ISA-51 VG		MONTAN	Purchased, study funded
Sargramostim (GM-CSF)	Leukine	GM-CSF	Purchased, study funded
PF-3512676 (CPG7909)		CP7909	Pfizer Pharm, Inc.

1.0 Background

1.1 Breast cancer is diagnosed in 200,000 individuals in the United States annually and contributes to approximately 40,000 deaths each year. For tumors confined to the breast, surgical removal provides a good prognosis. However, primary tumor that metastasizes to distant sites, such as lymph nodes, lungs, liver and brain, correlates with a poor prognosis. Patients with advanced stage breast cancer are at high risk of relapse. Complications from metastatic disease are the leading causes of cancer-related deaths. Novel adjuvant strategies, such as breast cancer specific vaccines, are being considered as a clinical intervention that may reduce the chance of recurrence.

In recent years there has been great interest in the development of these cancer vaccines, which are designed to immunize individuals to antigens present on tumors. Cancer vaccines are a non-toxic therapy, which have been shown in several melanoma trials to have the potential of controlling disease and prolonging survival because tumors can be surgically removed and there is often a long period of time before the tumor recurs at metastatic sites, cancer vaccines have been proposed as an optimal therapy that could prolong the time to recurrence. This optimal opportunity of immunization in the situation of minimal residual disease has rarely been tested, however, as most vaccines have been given to patients with large tumor burden after the failure of standard therapies in Phase I and Phase II trials. Recently, several groups have addressed the use of adjuvant immunotherapy following complete surgical resection [1]. Data from these studies are not yet complete.

1.2 The past two decades in tumor immunology have led to the discovery of specific tumor antigens that have been shown in preclinical studies to elicit tumor-specific immunity and establish long term memory without autoimmunity. For breast cancer, vaccines composed of epitopes derived of MUC1, HER-2/neu, MAGE3, CEA have been studied and shown to be immunogenic without causing autoimmunity [2-5].

1.3 It is now clear that tumor antigens are presented in the context of specific class I and Class II HLA molecules. Class I presentation, in the presence of appropriate co-stimulation, is thought to stimulate a cytolytic $CD8^+$ T cell response, while antigen presentation in the context of Class II molecules stimulates a $CD4^+$ helper T cell response [6].

1.4 One approach for the development of a cancer vaccine is the use of tumor associated synthetic antigens for immunologic priming. Because specific peptides are ubiquitous in tumors of the same histologic type, identical peptide vaccines may be employed in allogeneic hosts bearing the same tumor histology. Additionally, the use of single peptides for immunization limits the potential induction of undesired autoimmunity [7-9]. Recent developments in the use of soluble MHC Class I/peptide tetramers and elispot technology have enabled rapid characterization of epitope-specific CTL responses [10, 11]. In addition to being well-explored and understood, many of these antigens are shared tumor antigens. Vaccines that are composed of these antigens can be developed for use in a large number of patients. The primary limitations to peptide based vaccine strategies are haplotype restriction, potential for degradation, and uncertainty regarding which peptides, used alone or in combination, are the most immunogenic [12, 13]. This study is designed to test these uncertainties.

1.5 One attractive and broadly applicable target for immunotherapeutic strategies is the MUC1 tumor antigen. MUC1, a cell-associated mucin, is expressed on the cell surface of many epithelial malignancies as well as by hematological malignancies [14-17]. These include multiple myeloma (92%) and acute myelogenous leukemia (67%) [18]. Greater than 90% of breast carcinomas express MUC1; high levels are also found in adenocarcinomas originating from most tissues [14, 16]. MUC1 expression is greatly up-regulated on tumors (reviewed in

Gendler [19]). Expression on tumors is no longer apical, but it is found all around the cell surface and in the cytoplasm. In addition, glycosylation on tumor-synthesized MUC1 is aberrant, with greater exposure of the peptide core than is found in normal tissues. MUC1 has long been an interesting target molecule for immunotherapeutic strategies, given its high level and ubiquitous expression. Patients with tumors, especially with breast, pancreas and ovarian tumors, have exhibited immune responses to MUC1 with the presence of antibodies and T cells specific for MUC1 detected in about 10% of individuals. An HLA unrestricted T cell response among cancer patients has also been described [20-23]. There is increasing evidence from murine and human studies that MHC-restricted T cells can be induced in mice and humans after immunization with the MUC1 peptide or MUC1 antigenic epitopes [24-32]. Importantly, there have been reports of two HLA-A2 binding peptides derived from the MUC1 protein [33]. One of the peptides is from the tandem repeat sequence of MUC1 and the second peptide is from the signal sequence. MUC1-specific cytotoxic T cells (CTLs) have been induced in T cells from healthy donors following *in vitro* immunization using peptide-pulsed dendritic cells. MUC1-specific CTLs have also been induced *in vivo* after vaccination of breast and ovarian cancer patients with peptide-pulsed DCs [18].

1.6 A second candidate for peptide-based immunotherapy is HER-2/neu, the gene product of the erbB2/neu protooncogene. HER-2/neu is overexpressed in approximately 30% of breast cancer patients. HER-2/neu is also expressed by multiple types of tumors, including ovarian, lung, colon, pancreas and gastric tumors [34-36]. HER-2/neu has particular relevance, as it is expressed at high levels in early *in situ* lesions in breast carcinoma [37]. Thus, it is a target for early disease. Immunologic responses to HER-2/neu have been detected in a minority of patients with advanced stage breast and ovarian cancer, including antibodies, T helper and CD8 responses [38, 39]. Several HLA-class I binding peptides have been previously identified. A novel HLA-A2.1 binding peptide from the HER-2/neu extracellular domain [HER-2(9_{435})] was recently identified [40]. This peptide (ILHNGAYSL) bound to HLA-A2.1 with intermediate affinity (IC50 74.6 nM). The HER-2(9_{435}) epitope was tested using an *in vitro* immunization protocol and found to elicit CTLs that killed peptide-sensitized target cells. The CTLs elicited also recognized the HER-2/neu antigens, as it specifically killed tumor cells expressing the HLA-A2.1 and HER-2/neu antigens (see below in preliminary data). Furthermore, recognition of the tumor cell targets was significantly inhibited by unlabeled (cold) targets pulsed with HER-2(9_{435}), but not by unlabeled targets either unpulsed or pulsed with a control HLA-A2.1 binding peptide (see below). Thus, the CTLs induced by HER-2(9_{435}) are antigen specific.

A potential limiting factor for peptide based immunotherapy is related to a defined antigenic repertoire which is HLA restricted. This factor, inherent to all peptide-based approaches, restricts patient access. Additionally, because individual peptides only have the potential to induce epitope-specific CTL, the vast majority of potential tumor antigens are not targeted. In this setting, tumor down regulation of individual antigens or HLA epitopes promotes immune evasion. Recent evidence, however, suggests that this problem of epitope restriction may not be as physiologically important as was previously postulated. Specifically, it has now been clearly demonstrated that a T cell response induced against one epitope can stimulate CTL response to other target epitopes through a mechanism termed epitope spreading [3, 41, 42]. Using an experimental autoimmune encephalitis model, Vanderlugt et al. have demonstrated that disease progression is associated with the development of epitope-specific helper T cells, which are distinct from those initiating the disease. Transfer of secondary $CD4^+$ cells to naïve mice induces the disease phenotype and the disease is abrogated by blocking the secondary T cell response even though the primary T cell response remains intact [43, 44]. Disis demonstrated epitope spreading in 84% of patients vaccinated with HER-2/neu peptides, reflecting the initiation of an endogenous immune response. The immunity persisted after active immunizations ended [3]. These data suggest that peptide based approaches to cancer immunotherapy may indirectly stimulate multiple tumor reactive CTL against minor antigens in the presence of residual tumor. Based on this concept, the current study is designed as a therapeutic approach, with peptide epitope selection designed to enhance the number of potential candidates.

In addition to class I epitopes, immunogenic HLA-DR restricted class II epitopes have been defined for HER-2/neu. $CD4^+$ helper T lymphocytes (T_H) responses play an essential role in immunologically mediated anti-tumor immunity [45]. T_H lymphocytes provide CTLs with growth-stimulating cytokines, prime/activate DCs to effectively present antigen to naive CTL precursors [46-48] and they are important in the development of immune memory [49-51]. The development of IgG antibodies to HER-2/neu and the identification of $CD4^+$ T cells that secrete cytokines in response to HER-2/neu peptides or recombinant HER-2/neu protein suggest responses to helper T cells [52-57]. A promiscuous MHC class II T_H epitope has been identified for the HER-2/neu antigen (HER-2_{883}). T cell responses are restricted by HLA-DR1, HLA-DR4, HLA-DR52, and HLA-DR53 [58]. Peptide-induced T cells were effective in recognizing naturally processed HER-2/neu protein. The peptide HER-2_{883}, (KVPIKWMALESILRRRF), which was selected by computer algorithm, was tested for its capacity to stimulate $CD4^+$ T cells isolated from four healthy, MHC-typed individuals (DR1/11, DR1/13, DR4/15, DR7/17) in primary *in vitro* culture using peptide pulsed autologous DCs. T cells that proliferated were found to react with peptide and recombinant HER-2/neu intracellular domain protein presented by autologous DCs (see below). These results, showing reactivity with recombinant protein, suggest that HER-2_{883} is naturally processed, as the peptide stimulated T cells react with DCs primed with recombinant protein. Clearly, HER-2_{883} is a naturally processed peptide epitope and is promiscuous for multiple HLA-DR epitopes, making it an ideal candidate for therapeutic applications.

1.7 Because of the expression of MUC1 and HER-2/neu in multiple cancers, the development of this peptide-based immunotherapy can potentially impact the treatment of multiple disease entities, not only adenocarcinomas but hematopoietic malignancies as well. There is considerable interest in the use of the MUC1 peptide vaccination for treatment of multiple myeloma following transplant when there is minimal residual disease prior to remission.

1.8 GM-CSF

Granulocyte-macrophage colony stimulating factor (sargramostim, GM-CSF) is a commercially available cytokine currently used in patients undergoing chemotherapy to shorten the duration of post-chemotherapy neutropenia. Recently published evidence also suggests that GM-CSF may play a role as an immune adjuvant [59, 60]. The following observations illustrate the mechanisms by which GM-CSF can potentiate the immunogenicity of an antigen: 1) GM-CSF is a key mediator of dendritic cell (DC) maturation and function [61]; 2) GM-CSF increases surface expression of class I and II MHC molecules as well as co-stimulatory molecules of dendritic cells *in vitro* [61]; 3) GM-CSF enhances antibody responses to known immunogens *in vivo* [62]; 4) tumor cells transfected with genes encoding/expressing GM-CSF are able to induce long lasting, specific anti-tumor immune responses *in vivo* [63]; 5) GM-CSF encapsulated in biodegradable microspheres mixed with whole tumor cells resulted in systemic anti-tumor immune responses comparable to those of GM-CSF transfected tumor cells [64]. Therefore, addition of GM-CSF to an oligopeptide antigen may substantially enhance its immunogenicity.

In an attempt to optimally enhance the immunogenicity of the peptides we will deliver the antigens and GM-CSF emulsified in incomplete Freund's adjuvant (IFA, Montanide ISA-51). This delivery mechanism should be comparable to a previously demonstrated delivery mechanism utilizing GM-CSF suspended in microspheres and mixed with tumor cells (antigens). We hypothesize that the emulsified GM-CSF in close proximity to tumor antigen peptides will substantially enhance their immunogenicity. This proximity of antigen and GM-

CSF seems to be necessary for the adjuvant effect of GM-CSF, as systemic administration of equivalent doses in animal models has not demonstrated adjuvant activity. Also, the adjuvant/local inflammatory properties of IFA may play a role in attracting antigen presenting cells to the site of injection [53]. We have preliminary data demonstrating the plausibility of such a mechanism.

1.9a Preliminary data demonstrating the feasibility of this approach already exists. Rosenberg and investigators published effective generation of peptide-specific T cells in melanoma patients immunized with peptides derived from gp100 [65]. Despite the demonstration of a specific immune response, no clinical responses were detected. Addition of systemic GM-CSF resulted in more pronounced CTL and delayed type hypersensitivity reactions and in a few cases objective tumor regressions. Salgaller et al. utilized a peptide derived from the gp100 epitope suspended in IFA and demonstrated generation of specific T cell responses to the peptide in melanoma patients [66]. Both studies suggest that increased immunogenicity of the peptide antigens leads to a more pronounced T cell response, which in some cases results in a clinically relevant anti-tumor effect. In the proposed study, we will combine the immunoadjuvant effects of both IFA and GM-CSF with the goal of increasing the immunogenicity of the MUC1 and HER-2/neu immunodominant peptides.

Preliminary observations in an ongoing clinical study (MC9973) utilizing HLA-A2 specific melanoma differentiation antigen peptide vaccines in which the peptide is emulsified in a suspension of IFA and GM-CSF is demonstrating enhanced skin reactions if peptide emulsified in IFA is administered in the presence of GM-CSF. A dose of 50 mcg of GM-CSF in the presence of IFA and peptide results in extensive local skin reactions as well as evidence of a clinical response in one of seven patients thus far. No changes in the numbers of peptide specific CTLs were observed. However, a recent publication demonstrated superior numbers of vaccine specific CTLs generated in a peptide vaccine utilizing 225 mcg of GM-CSF in IFA) [67]. This would suggest a dose/response relationship of GM-CSF and anti-peptide vaccine CTL frequencies as determined by ELISPOT and tetramer assays. Therefore, in the current trial we propose to use 225 mcg of GM-CSF suspended in IFA (montanide ISA-51).

1.9b CpG (PF-3512676)

Therapeutic properties of bacteria in the treatment of malignant diseases (i.e. Coley's toxin) is an observation that has permeated the oncology literature for almost a century. More recently, it has been demonstrated that bacterial DNA possesses unique immunomodulatory features of potential utility in cancer therapy. Specifically, unmethylated CpG are able to stimulate NK cells and B cells. Furthermore, synthetic oligodeoxynucleotide (ODN) constructs containing unmethylated CpG motifs (CpG-ODN) were able to activate dendritic cells (DC) enhancing their antigen processing/presentation properties and stimulating production of Th1 cytokines necessary for CTL immune responses. Thus, CpG ODN appeared to function as an immune adjuvant. Several preclinical and clinical works illustrate the ability of CpG-ODN to function as a potent immune adjuvant for various forms of vaccines. One of the more interesting works, pertinent to this study, demonstrates the ability of CpG ODN to induce CTLs against a peptide vaccine when administered in conjunction with incomplete Freund's adjuvant (IFA) [68]. These authors used a MART-1/Melan-$A_{26\text{-}35}$ peptide emulsified in IFA with or without the addition of 50ug of CpG ODN to immunize human D^b (HHD) A2 transgenic mice. Their data suggest superior anti-peptide immunization in the CpG-ODN immunized group as determined by the frequency of tetramer positive CTLs. Our own data support these findings demonstrating superior immunization efficacy of IFA+CpG-ODN with ova peptide of C57BL/6 mice when compared to either IFA+peptide or complete Freund's adjuvant (CFA) + peptide (data not shown). An additional benefit to the CpG-ODN adjuvant is that it has been shown to be especially good at enhancing cellular and humoral immunity and promoting a Th1-type of response in older mice [69]. The population that develops cancer is mainly older individuals, thus the CpG-ODN adjuvant may be particularly relevant for this trial. Based on preclinical data suggesting the potent immune adjuvant properties of CpG co-emulsified with peptides in IFA, we elected to test the efficacy of CpG-ODN in the setting of a peptide vaccine immunization in this clinical trial. The dose of CpG-ODN that we decided to use in this study is 2mg/vaccine. The dose is based on published data demonstrating a direct dose-dependent relationship of CpG-ODN (0.125 –1 mg) and magnitude of measured immune responses (HepB vaccine adjuvant [70]). This is well below the highest tested doses of 20mg/week. Based on these observations we feel that the 2 mg dose is a reasonable starting point for a CpG-ODN adjuvant suspended in Montanide ISA 51 alone or in combination with GM-CSF.

1.9c Preliminary Data

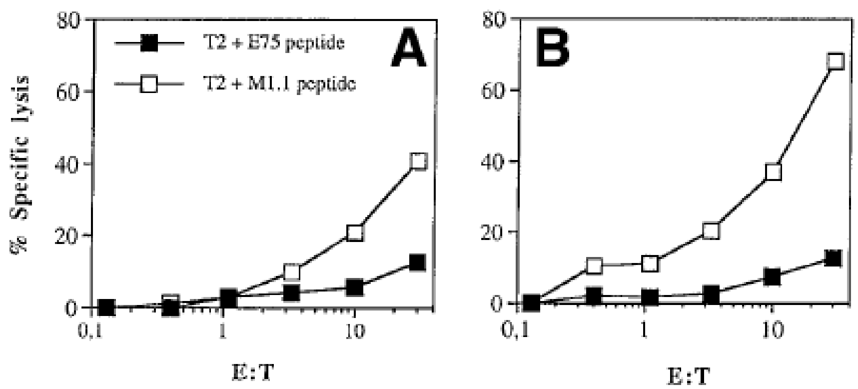
Preliminary data will be presented in multiple sections. First, we will provide data to support the choice of MUC1 and HER-2/erbB2 antigenic epitopes for this trial. Next, we will define our experience using peptides to stimulate tumor reactive T cells for cancer immunotherapy. Finally, we will discuss our experience with the immune adjuvants GM-CS and CpG-ODN. These preliminary data provide a strong foundation for the current proposal.

1.9c1 Identification of CTL Epitopes from MUC1

Using a computer analysis of the MUC1 amino acid sequence, two novel peptides were identified with a high binding probability to the HLA-A2 molecule [33]. Two peptides from MUC1 were identified; one from the tandem repeat M1.1 ($STAPPVHNV_{950-958}$) and one from the leader sequence M1.2 ($LLLLTVLTV_{12-20}$). The presence of the V in position 6 increases the binding of the M1.1 peptide to the HLA-A2 molecule. There is some variability in the tandem repeats in MUC1 and this sequence is found in the last tandem repeat. Cytotoxic T cells were induced from healthy donors by primary *in vitro* immunization using peptide-pulsed dendritic cells. The peptide-induced CTL lysed tumors endogenously expressing MUC1 in an antigen-specific and HLA-A2-restricted fashion..

+ PADRE peptide

CTL.M1.1



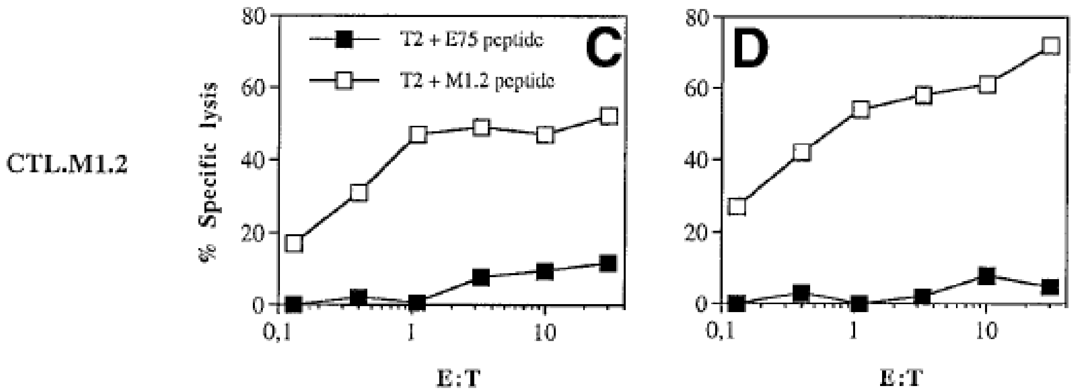


__Figure 1.__ Induction of CTL responses by peptide-pulsed dendritic cells. Adherent peripheral blood mononuclear cells were grown for 7 days with GM-CSF, IL-4, and TNF alpha. DCs pulsed with the synthetic peptides derived from the MUC1 protein (M1.1 and M 1.2) were used to induce a CTL response in vitro. In addition to the MUC1 peptide DCs were incubated with the PAN-DR binding peptide PADRE as a T-helper epitope. Cytotoxic activity of induced CTL was determined in a standard ^{51}Cr-release assay using T2 cells as targets pulsed for 2 hours with 50 μg of the cognate (open symbols) or irrelevant HER-2/neu protein-derived protein derived E75 peptide (solid symbols). (data reproduced from Brossart 1999 [33])

Next, the ability of the induced MUC1-specific CTL lines to lyse tumors expressing MUC1 was tested. MCF-7 cells that express MUC1 endogenously and are HLA-A2 positive were used as targets in a standard ^{51}Cr-release assay. The controls were SK-OV-3 cells, which express MUC1, but are HLA-A2 negative and the immortalized B cell line, Croft, which is A2 positive and was pulsed with MUC1 M1.1 or M1.2 peptides or the irrelevant HER-2/neu E75 peptide.

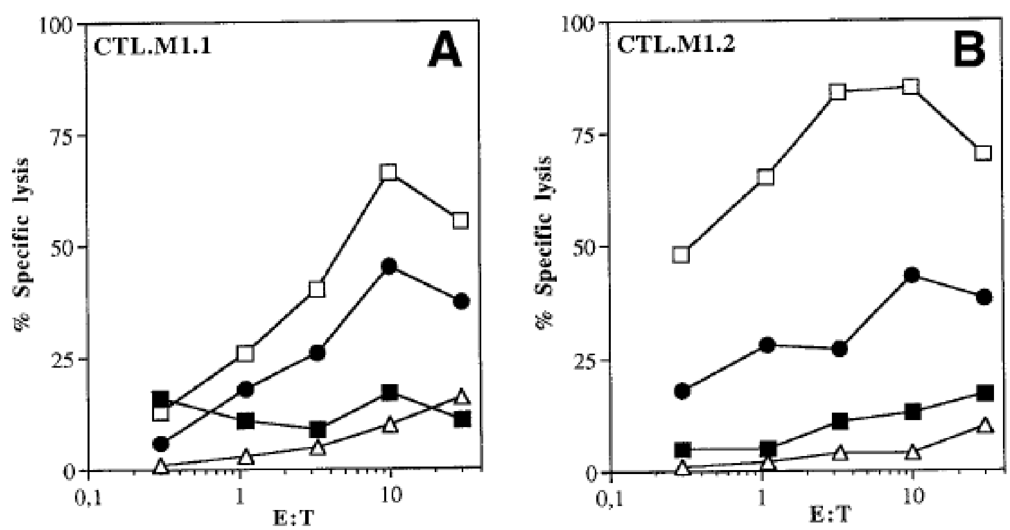
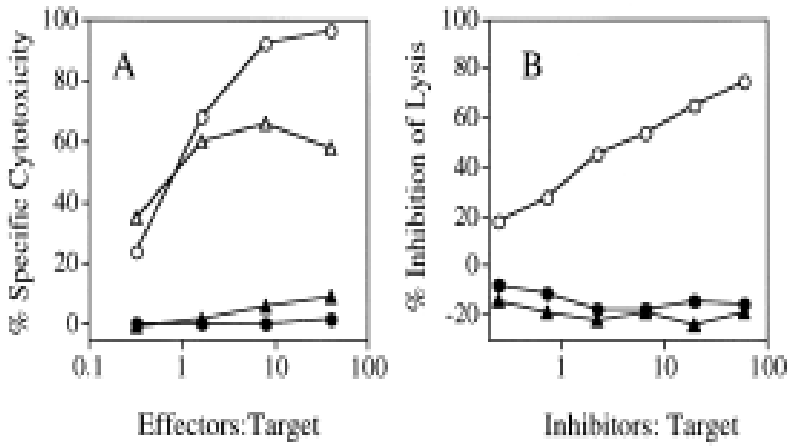


__Figure 2.__ Lysis of cancer cells endogenously expressing MUC1 by CTL.M1.1 (A) and CTL.M1.2 (B). Human breast cancer cell line MCF-7 (HLA-A2^{+}/MUC1^{+}), ovarian cancer cell line SK-OV-3 (HLA-A2^{-}/MUC1^{+}), and the immortalized B-cell line Croft (HLA-A2^{+}/MUC1^{-}) were used as targets in a standard ^{51}Cr-release assay. Croft cells were pulsed with the MUC1 peptides or an irrelevant HER-2/neu-derived peptide E75. (■) Croft + E75 peptide; (□) Croft + M1.1 (A) or M1.2 (B); (●)MCF-7; (Δ) SK-OV-3.

We have chosen to use the M1.1 peptide based on the large amount of data on the response to the MUC1 tandem repeat peptide, both in the human situation as well as in the mouse. Obviously only the human data are relevant for the clinical trials. We will use a HER-2/neu helper epitope (see below, not the PADRE helper epitope)

In the case of HER-2/neu, we have identified a novel CTL epitope HER-2 (9_{435}), which bound HLA-A2.1 with intermediate affinity (IC_{50} 74.6 nM). The peptide identified is: ILHNGAYSL. The .221(A2.1) cell line, produced by transferring the HLA-A2.1 gene into the HLA-A, -B, -C null mutant human lymphoblastoid cell line .221, was used as target (peptide loaded) to measure activity of HLA-A2.1 restricted CTL [71]. The CTLs elicited following in vitro stimulation effectively killed HLA-A2.1^{+} tumor cells, showing that the antigen is appropriately processed by tumors (Fig. 3A). In addition, recognition of the tumor cell target was significantly inhibited by unlabeled (cold) target pulsed with HER-2 (9_{435}) peptide, but not by unlabeled targets either unpulsed or pulsed with a control HLA-A2.1 binding peptide (Fig. 3B).



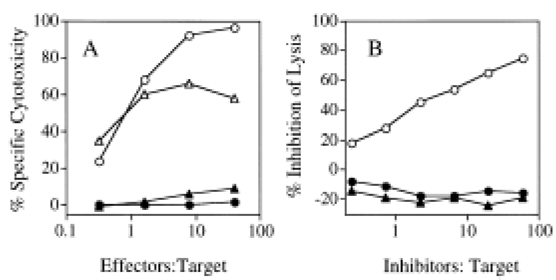


Figure 3. *HER-2(9_{435}) specific CTL can kill tumor cells. (A) HER-2(9_{435}) specific CTL were used as effector cells to test for the lysis of the following target cell lines: o, .221A2.1 pulsed with HER-2(9_{435}); ●, .221A2.1 without peptide; Δ, SW403 (colon CA, A2^{+}, HER-2/neu^{+}); ▲, HT-29 (colon ca, A2^{-}, HER-2/neu^{+}). (B): Antigen specificity demonstrated by cold target inhibition assay. Lysis of ^{51}Cr labeled SW403 cells at an effectors/target ratio of 10:1 by the HER-2(9_{435}) specific CTL was blocked at various Inhibitors/Target ratios by the following cold targets: o, .221A2.1 pulsed with HER-2(9_{435}); ▲, .221A2.1 pulsed with irrelevant A2.1 binding peptide (HBc_{18-27}); ●, .221A2.1 without peptide.*

In addition to the class I epitopes described above, a promiscuous MHC class II epitope was defined for HER-2/neu using the algorithm tables published by Southwood et al. [58, 72]. The epitope identified is HER-2_{883} (KVPIKWMALESILRRRF). It is important to show that these peptides represent true T cell epitopes that are relevant for the development of tumor immunotherapy. For these experiments autologous PBMCs or DCs were used as APCs and recombinant DNA derived intracellular domain or extracellular domain protein fragments of HER-2/neu were used as a source of antigen. The data in Fig. 4 show that four HER-2_{883}-reactive T cell lines proliferated well to HER-2/neu intracellular domain protein, which encompasses the HER-2_{883} peptide but not to HER-2/neu extracellular domain (ECD), which lacks HER-2_{883}..

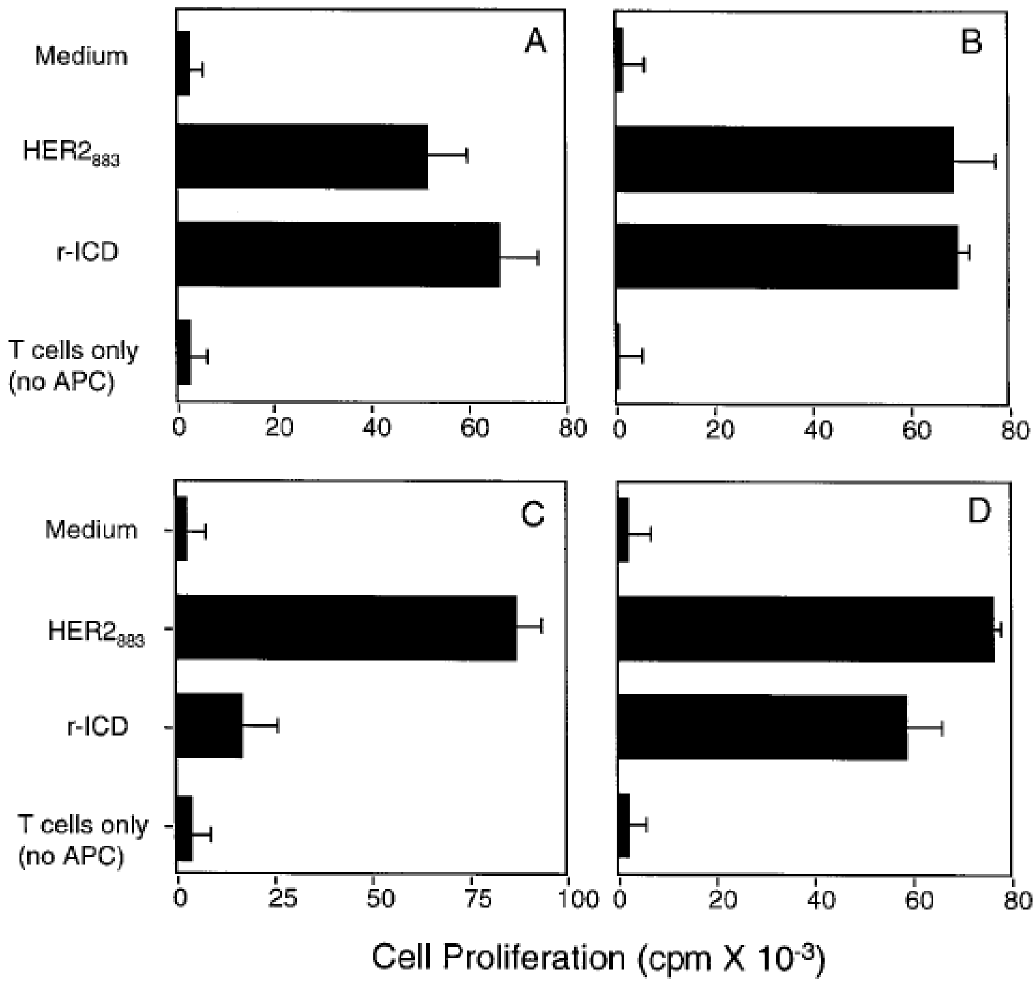


Figure 4. *HER-2_{883}-specific CD4+ T cells can recognize recombinant HER-2/neu intracellular domain (r-ICD) protein presented by autologous Dcs in the context of several HLA-DR alleles. The HER-2_{883}-reactive HTLs, TCL-7C (panel A, HLA-DR53 restricted), TCL-6D (panel B, HLA-DR4-restricted), a clone of TCL-1D (panel C, HLA-DR52-restricted), and TCL-1E (panel D. HLA-DR53 restricted), were tested for their capacity to proliferate to autologous DCs in the presence of HER-2_{883} peptide (2.5 mg/ml) or recombinant HER-2/neu recombinant ICD protein (10 mg/ml). No significant proliferative response was observed against HER-2/neu ECD protein (data not shown). Values shown are the means of triplicate determinations; bars, SD.*

1.9d Justification of vaccination strategy

1.9d.1 **Peptide dose** (1000 mcg): Over the last several years there has been extensive debate over the optimal dose of peptide in a variety of peptide immunization cancer clinical trials. Peptide doses have ranged from 50 mcg to 2500 mcg in various studies. Currently, the largest peptide vaccine clinical trial (E4697) utilizes a peptide dose of 1000 mcg. There are several published studies evaluating peptide vaccine dose-responses [66, 73], suggesting that 1000 mcg of peptide would be a reasonable vaccine dose for phase I/II clinical testing.

1.9d.2 **GM-CSF suspended in Montanide ISA 51 as a vaccine adjuvant.** The utility of GM-CSF suspended in montanide ISA 51 as an effective vaccine adjuvant has already been demonstrated in pre-clinical and clinical studies. Our own pre-clinical data (Fig 5) demonstrates a bell shaped dose-response curve for GM-CSF co-emulsified with 10 mcg of *ova* peptide in montanide ISA 51. Two weeks after immunization, the optimal dose of GM-CSF in the mouse model appears to be 100 mcg. In humans, Slingluff et al. demonstrated successful peptide immunization using 225 mcg of GM-CSF suspended in montanide ISA-51[67]. Up to 80% of treated patients demonstrated effective immunization with melanoma differentiation antigen peptides. Our clinical data using 10, 50, 75 and 100 mcg of GM-CSF suspended with peptides in Montanide ISA-51 failed to demonstrate effective generation of anti-peptide CTLs. In view of these data, we felt that it was reasonable to utilize the same dose of GM-CSF used by Slingluff [67] (225 mcg) with our current set of peptides. If successful, further studies will be performed attempting to generate a dose-response curve of GM-CSF and immunization efficacy similar to that of the mouse model.

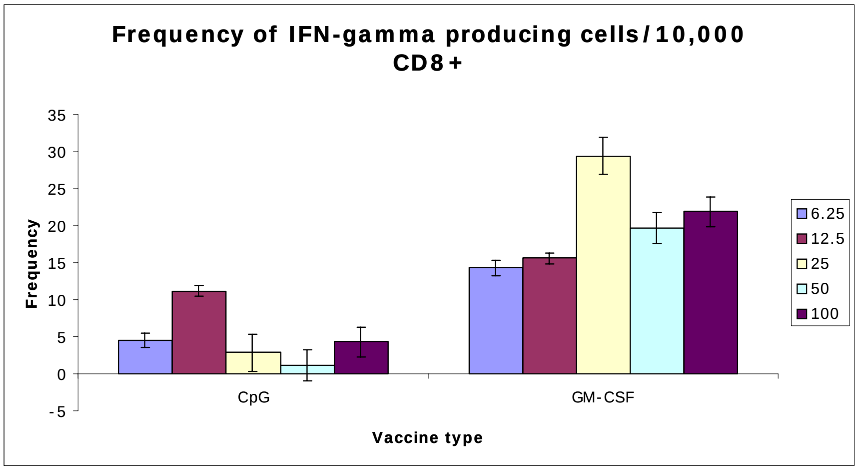


Fig. 5: C57BL6 mice (3 per group) were immunized with 10ug of ova peptide suspended in Montanide ISA51 and varying concentrations of CpG or GM-CSF .Represented are the frequencies of ova specific CTLs (IFN gamma ELISPOT) isolated from splenocytes on day 12 post immunization. Similar dose/response curves were observed in two other experiments.

1.9d.3 **CpG suspended in Montanide ISA 51 as vaccine adjuvant.** As described in section 15.7, the co-emulsification of peptide antigens with CpG and Montanide ISA-51 is an effective means of generation of peptide specific CTLs in a pre-clinical model. Our own data confirm these findings using non-transgenic mice immunized with ova peptide co-suspended with CpG in Montanide ISA 51 (Fig. 5). The dose of CpG used in the current study was empirically selected based on the results of a phase I clinical trial utilizing CpG (abbreviated as ISS in Fig. 6 legend) as an immune adjuvant for hepatitis B vaccine immunization in healthy volunteers. In this study, volunteers were immunized with an intramuscular injection of hepatitis B vaccine (20 mcg) mixed with CpG in one of the following amounts: 225 mcg, 650 mcg, 1000 mcg or 2250 mcg. A booster injection was administered 2 months later. Serologic data demonstrated (Fig 6) maximal immunization efficacy at CpG doses between 1000 and 2250 mcg. Based on these data suggesting a bell-shaped dose response curve for CpG (optimum may be between doses 1000 mcg and 2250 mcg) as well as our pre-clinical bell-shaped dose response curve, we elected to proceed with a CpG dose of 2000 mcg.

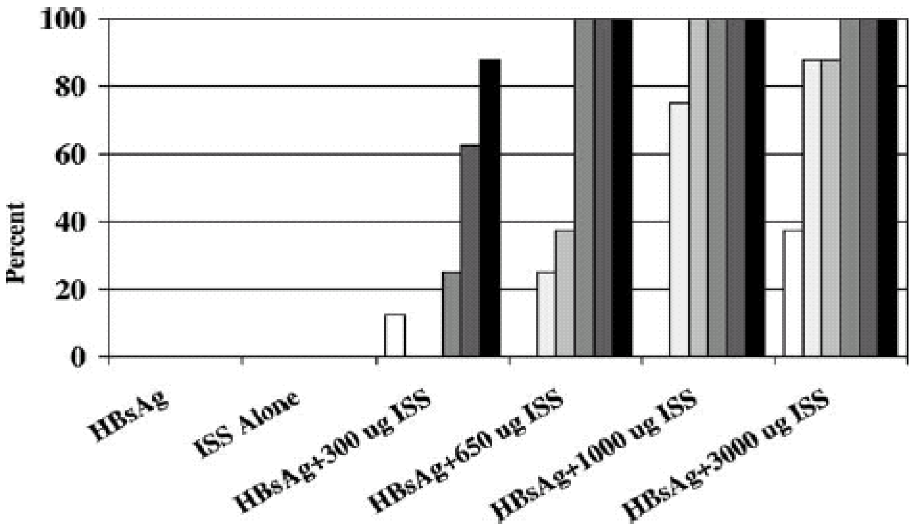


Figure 6: Proportion of participants achieving a protective antibody level (≥10mIU/mL) at various time points after immunization. Time points are (by increasing darkness of bar shade) 7 days after dose 1, 28 days after dose 1, 56 days after dose 1, 7 days after dose 2, 4 months after dose 2. CpG is designated as ISS.

The target population for this clinical trial, to whom the study findings will be generalized, are patients with a history of completely treated stage II or III breast adenocarcinoma that is MUC1 positive, currently off active therapy (with the exception of hormonal therapy) with no evidence of tumor relapse.

1.9e As of February 24, 2009, adverse event data are available for 4 patients randomized to Arm A, and 4 patients randomized to Arm B, and 5 patients randomized to Arm C. One patient on Arm A developed a grade 2 injection site reaction (ISR) during the first cycle of treatment and 2 patients on Arm B developed a grade 2 ISR during the third cycle of treatment. Three of the 5 patients randomized to Arm C also developed ISRs, namely, a grade 2 ISR during the first cycle of treatment which worsened to a grade 3 during the second cycle of treatment, a grade 2 ISR during the first and second cycles of treatment, and a grade 2 ISR during the third cycle of treatment. These ISRs were the only treatment-related toxicities reported. As the regimens containing CpG appear to induce a greater numbers of ISR, the dose of CpG will be lowered to 1 mg and all patients who develop a grade 2 ISR will discontinue study treatment.

2.0 Goals

2.1 Primary Goal

To determine the safety and immunization efficacy of MUC1 and HER-2/neu peptide vaccines combined with CpG, GM-CSF or both, as immune adjuvants suspended in Montanide ISA-51.

2.2 Secondary Goal

To describe the impact of immunization on clinical outcomes in patients with MUC1 positive breast cancer. Clinical outcomes of interest will include: (1) **disease-free survival** defined as the time from registration to the documentation of a first failure where a failure is the recurrence (REC) of breast cancer or a diagnosis of a second primary cancer (NEWP); and (2) **overall survival** defined as the time from registration to death due to any cause.

3.0 Patient Eligibility

3.1 Pre-registration – Inclusion Criterion

3.11 Central pathology review submission. This review for MUC1 positivity is mandatory prior to registration to confirm eligibility (see Section 17.0). **It should be initiated as soon as possible after pre-registration.**

3.2 Registration - Inclusion criteria

3.21 Age ≥18 years.

3.22 Completed "standard first line therapy ONLY" (including adjuvant therapy) for breast cancer, clinical stage II and III (≥3 months prior to registration) and currently with no evidence of disease. NOTE: Current use of "anti-estrogen" therapy is allowed. Patients with stage I breast cancer with "high-risk" features, including any of the following, are also eligible for enrollment if other entry criteria are met: HER2 over-expression or amplification, "triple-negative" (i.e., no expression of ER, PR, or over-expression of HER2 on routine immunohistochemical staining).

3.23 Histologically confirmed adenocarcinoma of the breast treated with surgery, adjuvant chemotherapy, and/or radiation therapy.

3.24 MUC1 positive breast cancer as determined by pre-registration central pathology review.

3.25 HLA-A2 positive.

3.26 The following laboratory values obtained ≤14 days prior to registration:

- Hemoglobin ≥8.0 g/dL
- Platelets ≥75,000/μL
- ANC ≥1,500/μL
- Creatinine ≤2 x ULN
- AST ≤2 x ULN

3.27 Capable of understanding the investigational nature, potential risks and benefits of the study and capable of providing valid informed consent.

3.28 Willingness to return to Mayo Clinic Rochester, Scottsdale, or Jacksonville for treatment and study-related follow up. Study treatment will be administered only at the Mayo Clinic site where the patient was enrolled. Post-treatment study follow-up is allowed at the other participating Mayo Clinic sites.

3.29a Willingness to provide the blood specimens and complete the imaging studies as required by the protocol.

Note: The goals of this study include assessment of the biologic effects on surrogate markers of the agent(s) being tested and are, therefore, contingent upon availability of the blood specimens and completion of the required imaging studies.

3.29b Negative serum pregnancy test done ≤7 days prior to registration, for women of childbearing potential only.

3.3 Registration - Exclusion criteria

3.31 ECOG performance status (PS) 3 or 4 (see Appendix I).

3.32 Uncontrolled infection.

3.33 Any of the following:
- Known HIV infection
- Other circumstances (i.e. concurrent use of systemic immunosuppressants and immunocompromising condition) that in the opinion of the physician renders the patient a poor candidate for this trial

3.34 Failure to fully recover from acute, reversible effects of prior breast cancer therapy regardless of interval since last treatment.

3.35 Any of the following:
- Pregnant women
- Nursing women unwilling to stop breast feeding
- Women of childbearing potential who are unwilling to employ adequate contraception (diaphragm, birth control pills, injections, intrauterine device [IUD], or abstinence, etc.)

NOTE: This study involves an investigational agent whose genotoxic,mutagenic and teratogenic effects on the developing fetus and newborn are unknown.

3.36 Other concurrent chemotherapy, immunotherapy, radiotherapy, or any ancillary therapy considered investigational (utilized for a non-FDA-approved indication and in the context of a research investigation).

3.37 Radiographic evidence of disease at the time of enrollment.

3.38 Any prior invasive malignancies ≤5 years (with the exception of curatively-treated basal cell or squamous cell carcinoma of the skin or carcinoma in situ of the cervix).

3.39 Primary surgery for breast cancer **beyond 3 years** at time of registration.

4.0 Test Schedule

Tests and procedures	Active Monitoring Phase				
	Pre-Reg	≤14 days prior to reg	Prior to each subsequent treatment (q 4 weeks)	At 4 weeks after last treatment	Observation q 3 months until disease recurrence or for maximum of 2 years following registration
Central pathology review (see Section 17.1) [5]	X				
History and assessment, wt, PS		X	X[R]	X	X
Height		X			
Hematology group: WBC, ANC, Hgb, PLT		X[R]	X[8]	X[R]	X
Chemistry group: total and direct bilirubin, AST, creatinine		X[R]	X[8]	X[R]	X
HLA class I and II typing [R]		At any time prior to reg			
Serum pregnancy test [1]		X			
Tumor typing [R]		At any time prior to reg			
Tumor evaluation by imaging study (x-ray, CT or PET)		X			X[2]
DTH skin testing (common recall antigens) [3, R]		X	Prior to cycle 6 only		
Research blood specimens [7] See section 14.0		X	X[4]		X[4]
Acute toxicity evaluation [6]			X	X	

1. For women of childbearing potential, must be obtained ≤7 days prior to registration.
2. Imaging will be performed per “standard of care” for patients and at the discretion of the treating physician
3. DTH skin testing will be performed using the same complement of antigens in routine use at the treatment site.
4. Research blood samples will be performed prior to registration, prior to cycles 3, 5 and 6 of therapy as well as every 3 months after conclusion of active therapy until 24 months following registration.
5. Tumor tissues will be stained for MUC1 and HER-2/neu mandatory central review after pre-registration but prior to registration.
6. Acute toxicity evaluations (physical exam and laboratory testing) will be performed for the purpose of evaluating potential immediate side effects of immunization.
7. Research blood specimens will be collected only if serum hemoglobin for the given collection is ≥10 g/dL. If hemoglobin is <10 g/dL, research blood samples will be postponed until the next study office visit.
8. Research funded prior to cycles 2 and 5.

R. Research Funded

5.0 Stratification Factors (*collected at registration*)

Her-2/neu status: Positive vs. negative

6.0 Registration/Randomization Procedures

6.1 Pre-Registration (Step 1)

6.11 To pre-register a patient, access the Mayo Clinic Cancer Center (MCCC) web page and enter the remote registration/randomization application. The remote registration/randomization application is available 24 hours a day, 7 days a week. Back up and/or system support contact information is available on the Web site. If unable to access the Web site, call the MCCC Registration Office at (507)-284-4130 between the hours of 8 a.m. and 4:30 p.m. Central Time (Monday through Friday).

The instructions for remote pre-registration are available on the MCCC web page (http://hsrwww.mayo.edu/ccs/training) and detail the process for completing and confirming patient pre-registration. Users should refer to the section titled "Pre-Registration Components" for details on how to pre-register a patient to a study. At the time of pre-registration the patient will receive a MCCC patient identification number. This number is to be used when submitting tissue or blood samples, if applicable for the study (See Sections 14.0 and/or 17.0). Patient pre-registration via the remote system can be confirmed in any of the following ways:

- Contact the MCCC Registration Office (507)-284-4130. If the patient was pre-registered, the Registration Office staff can access the information from the centralized database and confirm the pre-registration.
- Refer to "Instructions for Remote Registration" in section "Finding/Displaying Information about A Registered Subject."

6.12 Prior to accepting the registration/randomization, the remote registration/randomization application will verify the following:

- IRB approval at the registering institution
- Patient eligibility
- Existence of a signed consent form
- Existence of a signed authorization for use and disclosure of protected health information

6.2 Registration (Step 2)

6.21 To register a patient, access the Mayo Clinic Cancer Center (MCCC) web page and enter the remote registration/randomization application. The remote registration/randomization application is available 24 hours a day, 7 days a week. Back up and/or system support contact information is available on the Web site. If unable to access the Web site, call the MCCC Registration Office at (507) 284-2753 between the hours of 8 a.m. and 5:00 p.m. Central Time (Monday through Friday).

The instructions for remote registration are available on the MCCC web page (http://hsrwww.mayo.edu/ccs/training) and detail the process for completing and confirming patient registration. Prior to initiation of protocol treatment, this process must be completed in its entirety and a MCCC subject ID number must be available as noted in the instructions. It is the responsibility of the individual registering the patient to confirm the process has been successfully completed prior to release of the study agent. Patient registration via the remote system can be confirmed in any of the following ways:

- Contact the MCCC Registration Office (507) 284-2753. If the patient was fully registered, the Registration Office staff can access the information from the centralized database and confirm the registration.
- Refer to "Instructions for Remote Registration" in section "Finding/Displaying Information about A Registered Subject."

6.22 A mandatory translational research component is part of this study. The patient will be automatically registered onto this component (Section *14.0)*.

6.23 A signed HHS 310 form must be on file in the Registration Office before an investigator may register any patients. Ongoing approval documentation must be submitted (no less than annually) to the Registration Office.

6.24 Prior to accepting the registration/randomization, the remote registration/randomization application will verify the following:
- IRB approval at the registering institution
- Patient eligibility

6.25 Treatment on this protocol must commence at Mayo Clinic Rochester, Scottsdale or Jacksonville under the supervision of a medical oncologist or hematologist.

6.26 Treatment cannot begin prior to registration and must begin ≤7 days after registration.

6.27 Pretreatment tests/procedures must be completed within the guidelines specified on the test schedule.

6.28 All required baseline symptoms must be documented and graded.

6.29 Study drug availability checked.

7.0 Protocol Treatment

7.1 For the purposes of this trial, patients will be recruited from the breast cancer practice of the Mayo Clinic Cancer Center. Patients who are undergoing regular follow-up visits by Mayo Clinic oncologists, are interested in this study, and fulfill all eligibility criteria will be offered enrollment. The patients' primary physicians, co-investigators in this study, will have the opportunity to offer the study to interested patients during their regularly scheduled follow-up visits. It is not expected that recruitment or advertisement materials will be used.

The patients who are enrolled will be assigned a 'study number' which will be used for their identification, and that of their data, throughout their participation in the clinical trial.

The Informed Consent process will take place during the patient's regular follow-up visits with their oncologists, co-investigators in the clinical trial. The informed consent interview will begin as part of the patient's regular follow-up visit. At that time, interested patients will be given information about the study, and if interested, will also receive a copy of the Informed Consent document. Patients will have the opportunity to discuss the details of the study during this visit or, more likely, will be given the consent form and offered to review the document at home and schedule a follow-up visit if they are interested in taking part on the study. This way the patients will have a chance to investigate and discuss the study on their own. If interested, the patients will set-up a 2nd visit with their oncologists specifically for the purpose of deciding on study participation. At that visit, all issues of concern for the patient will be addressed, eligibility reviewed and, if appropriate, the Consent Form will be signed.

7.2 As part of the registration process described in Section 6.0, the Mayo Clinic Cancer Center (MCCC) Remote Registration application will assign patients to arms A through C.

7.3 Treatment Schedules:

Arm A

	Agent	Dose	Route	Rx Days	ReRx
Arm A	Montanide ISA-51	1.5 mL	subcutaneous injection in un-dissected LN region	Day 1 of Week 1	Q4 weeks (28-32 days) x 6 cycles
	MUC1 (STAPPVHNV)	1mg			
	HER-2 peptide 1 (ILHNGAYSL)	1mg			
	HER-2 peptide 2 (KVPIKWMALESILRRRF)	1mg			
	GM-CSF	0.225 mg			

Arm B

	Agent	Dose	Route	Rx Days	ReRx
Arm B	Montanide ISA-51	1.5 mL	subcutaneous injection in un-dissected LN region	Day 1 of Week 1	Q4 weeks (28-32 days) x 6 cycles
	MUC1 (STAPPVHNV)	1mg			
	HER-2 peptide-1 (ILHNGAYSL)	1mg			
	HER-2 peptide-2 (KVPIKWMALESILRRRF)	1mg			
	PF-3512676 (CPG7909)	1 mg			

Arm C

	Agent	Dose	Route	Rx Days	ReRx
Arm C	Montanide ISA-51	1.5 mL	subcutaneous injection in un-dissected LN region	Day 1 of Week 1	Q4 weeks (28-32 days) x 6 cycles
	MUC1 (STAPPVHNV)	1mg			
	HER-2 peptide-1 (ILHNGAYSL)	1mg			
	HER-2 peptide-2 (KVPIKWMALESILRRRF)	1mg			
	GM-CSF	0.225 mg			
	PF-3512676 (CPG7909)	1 mg			

7.4 Fifteen patients per arm (total of 45) will be randomly assigned to receive one of the three treatment schedules. Doses will not be escalated in any individual patient. It is not anticipated that there will be toxicity experienced with these regimens.

Vaccines will be administered as multiple (2-3) subcutaneous injections in regions of un-disturbed axillary or inguinal lymph nodes. Each vaccine cycle will be administered into a single lymph node draining area. Subsequent vaccination cycles will be administered to other (rotating) undisturbed lymph node drainage sites.

The main risks are those of an allergic reaction to the components of the peptide vaccine (local or systemic). To minimize risk, patients will be observed by a registered nurse for 30 minutes following each immunization. On-site physicians will be available in the unlikely event that complications do occur. Risks due to phlebotomy will be minimized by ensuring that all patients will undergo phlebotomy by certified phlebotomists. All patients will be provided detailed contact information so that they are able to contact their treating physicians/co-investigators if they experience problems (medical or otherwise) while undergoing therapy in this study.

There are no antidotes available for the peptide vaccines used in this protocol. If patients develop symptoms as a result of the vaccines (e.g. allergic reactions), those patients will be treated accordingly.

The benefit to patients who undergo treatment in this study is unknown.

As IND sponsor, the Principal Investigator will monitor the protocol in accordance with 21 CFR 312, as indicated in portions of section 4.0, the test schedule; section 10.0, the adverse event reporting; and section 15, the drug information.

8.0 Dosage Modification Based on Adverse Events - Adjustments are based on adverse events observed since the prior dose.

ALERT: *ADR reporting may be required for some adverse events (See Section 10)*

→→ ***Use Common Terminology Criteria for Adverse Events (CTCAE) v3.0 unless otherwise specified ←←***			
CTCAE CATEGORY	ADVERSE EVENT	AGENT	DOSAGE CHANGE OR OTHER ACTION
AT TIME OF RETREATMENT			
ALLERGY/ IMMUNOLOGY	≥Grade 2 allergic reaction/ hypersensitivity	Montanide GM-CSF CpG Peptides	Discontinue vaccinations indefinitely and begin event monitoring.
	≥Grade 2 autoimmune reaction (excluding vitiligo)		Discontinue vaccinations indefinitely and begin event monitoring.
Dermatology/Skin	≥Grade 2 injection site reaction		Discontinue vaccinations indefinitely and begin event monitoring
ALL OTHERS	≥Grade 3 Hematologic or ≥Grade 3 Nonhematologic (excluding alopecia) ≥Grade 2 neurologic		Discontinue vaccinations indefinitely and begin event monitoring.

9.0 Ancillary Treatment/Supportive Care

9.1 Patients should receive blood product support, antibiotic treatment and treatment of other newly diagnosed or concurrent medical conditions.

9.2 Patients participating in this clinical trial are not to be considered for enrollment in any other study involving a pharmacologic agent (drugs, biologics, immunotherapy approaches, gene therapy) whether for symptom control or therapeutic intent.

10.0 Adverse Event (AE) Reporting and Monitoring

10.1 This study will utilize the Common Terminology Criteria for Adverse Events (CTCAE) v3.0 for adverse event monitoring and reporting. The CTCAE v3.0 can be downloaded from the CTEP home page (http://ctep.info.nih.gov/CTC3/ctc_ind_term.htm). All appropriate treatment areas should have access to a copy of the CTCAE v3.0.

10.11 Adverse event monitoring and reporting is a routine part of every clinical trial. First, identify and grade the severity of the event using the CTCAE. Next, determine whether the event is expected or unexpected (refer to Section 15.0 and/or product literature) and if the adverse event is related to the medical treatment or procedure (see Section 10.12). With this information, determine whether an adverse event should be reported as an expedited report (see Section 10.2) or as part of the routinely reported clinical data.

Expedited adverse event reporting requires submission of a written report, but may also involve telephone notifications. Telephone and written reports are to be completed within the timeframes specified in Section 10.2. All expedited adverse event reports should also be submitted to the local Institutional Review Board (IRB).

10.12 Assessment of Attribution

When assessing whether an adverse event is related to a medical treatment or procedure, the following attribution categories are utilized:

Definite - The adverse event *is clearly related* to the investigational agent(s).
Probable - The adverse event *is likely related* to the investigational agent(s).
Possible - The adverse event *may be related* to the investigational agent(s).
Unlikely - The adverse event *is doubtfully related* to the investigational agent(s).
Unrelated - The adverse event *is clearly NOT related* to the investigational agent(s)

10.2 Expedited Adverse Event Reporting Requirements

Phase I, II and III Studies (Investigational)

	Grade 4 or 5[1] Unexpected with Attribution of Possible, Probable, or Definite	Other Grade 4 or 5 or Any hospitalization during treatment[6]	Secondary AML/MDS[2]
Notify the Cancer Center IND Coordinator [3] within 24 hours	X		
Submit written report within 5 working days[4]	X		
NCI/CTEP Secondary AML/MDS Report Form within 15 working days[5]			X
Submit Grade 4 or 5 Non-AER Reportable Events/Hospitalization Form within 5 working days.[6]		**X[6]**	

1. Includes all deaths within 30 days of the last dose of investigational agent regardless of attribution or any death attributed to the agent(s) (possible, probable, or definite) regardless of timeframe.
2. Reporting for this AE required during or after treatment.
3. Notify the Cancer Center IND Coordinator (Mayo Clinic - Rochester) by telephone (507) 284-0938 and/or submit a written event summary via fax to (507) 538-7164.
4. Use *Adverse Event Expedited Report – Single Agent* or *Multiple Agents* report form. Submit to the Cancer Center IND Coordinator (Mayo Clinic - Rochester) and to the Cancer Center Protocol Development Coordinator (PDC) for IRB reporting. The IND Coordinator will review the event in consultation with the IND holder and report to the Food and Drug Administration (FDA) as warranted by the event and required by U.S. federal regulations.
5. Submit per form-specified instructions and provide copy to Cancer Center IND Coordinator for review and FDA reporting (as warranted by the event) and the Cancer Center PDC for IRB reporting.
6. In addition to standard reporting mechanism for this type of event, submit information to the Cancer Center IND Coordinator and Cancer Center PDC. These persons will facilitate FDA and IRB reporting, respectively, as warranted by the event. If Adverse Event Expedited Report – Single Agent or Multiple Agents report form was completed, this form does not need to be completed.

10.3 Adverse events to be graded at each evaluation and pretreatment symptoms/conditions to be evaluated at baseline per Common Terminology Criteria for Adverse Events (CTCAE) v3.0 grading unless otherwise stated in the table below:

CTCAE Category	Adverse event/Symptoms	Baseline	Each evaluation
Constitutional Symptoms	Fatigue (Asthenia, lethargy, malaise)	X	X
Dermatology/Skin	Injection site reaction		X
	Rash/desquamation	X	X
Pain	Musculoskeletal - *Select*s • Bone	X	X
	• Joint	X	X
	• Muscle	X	X

10.31 Submit via appropriate MCCC Case Report Forms (i.e., paper or electronic, as applicable) the following AEs experienced by a patient and not specified in Section 10.3:

10.311 Grade 2 AEs deemed *possibly, probably, or definitely* related to the study treatment or procedure.

10.312 Grade 3, 4, and 5 AEs and deaths within 30 days of the patient's last treatment, regardless of attribution to the study treatment or procedure, with the exception of signs or symptoms of definitely related to the patient's disease or disease progression.

10.313 Any death more than 30 days after the patient's last study treatment or procedure which is felt to be at least possibly treatment related must also be submitted as a Grade 5 AE, with a CTCAE type and attribution assigned.

10.32 Refer to the instructions in the electronic data entry screens regarding the submission of late occurring AEs following completion of the Active Monitoring Phase (i.e., compliance with Test Schedule in Section 4.0).

Information included at the request of the Department of Defense, a financial sponsor of the study

Reporting of serious or unexpected adverse events and unanticipated problems.

a. Serious or unexpected adverse events and unanticipated problems can occur in any and all types of studies, not just experimental interventions or clinical trials.

b. Include a definition of what constitutes an adverse event in the study.

(1) For IND or IDE research include definitions as described in 21 CFR 312.32.

(2) All IND protocols must address the following requirements.

"An adverse event temporally related to participation in the study should be documented whether or not considered to be related to the test article. This definition includes intercurrent illnesses and injuries and exacerbations of preexisting conditions. Include the following in all IND safety reports: Subject identification number and initials; associate investigator's name and name of MTF; subject's date of birth, gender, and ethnicity; test article and dates of administration; signs/symptoms and severity; date of onset; date of resolution or death; relationship to the study drug; action taken; concomitant medication(s) including dose, route, and duration of treatment, and date of last dose."

c. Describe agencies or offices to be notified with point of contact information in the event of a serious and unexpected adverse event.

All protocols should contain the following language regarding the HSRRB reporting requirements for adverse events and unanticipated problems. (Note that unanticipated problems can occur in a study that does not require a research/clinical intervention.)

"Unanticipated problems involving risk to volunteers or others, serious adverse events related to participation in the study and all volunteer deaths should be promptly reported by phone (301-619-2165), by email (hsrrb@det.amedd.army.mil), or by facsimile
(301-619-7803) to the Army Surgeon General's Human Subjects Research Review Board. A complete written report should follow the initial telephone call. In addition to the methods above, the complete report can be sent to the U.S. Army Medical Research and Materiel Command, ATTN: MCMR-ZB-QH, 504 Scott Street, Fort Detrick, Maryland 21702-5012"

Refer to the "HSRRB Information Sheet for Investigators: Unanticipated Problems" for examples of unanticipated problems located on our website.

"The medical monitor for this project, Dr. Robert R. McWilliams, is required to review all unanticipated problems involving risk to volunteers or others, serious adverse events and all volunteer deaths associated with the protocol and provide an unbiased written report of the event. At a minimum the medical monitor should comment on the outcomes of the event or problem and in the case of a serious adverse event or death comment on the relationship to participation in the study. The medical monitor should also indicate whether he/she concurs with the details of the report provided by the study investigator. Reports for events determined by either the investigator or medical monitor to be possibly or definitely related to participation and reports of events resulting in death should be promptly forwarded to the HSRRB."

The medical monitor will forward reports to the U.S. Army Medical Research and Material Command, ATTN: MCMR-ZB-QH, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

11.0 Treatment Evaluation

11.1 For the purposes of this study, patients should be re-evaluated every 4 weeks during immunizations (treatment) and every 12 weeks during follow-up.

11.2 At the time of reevaluation, patients will be classified in the following manner:

11.21 No evidence of disease (NED).

11.22 Breast cancer recurrence (PD). Local/regional breast cancer recurrence is defined as the development of tumor (except LCIS) in the ipsilateral breast (after lumpectomy); in the soft tissue/chest wall and/or skin of the ipsilateral chest wall; or tumor in the ipsilateral internal mammary, infraclavicular, or axillary nodes or soft tissue of ipsilateral axilla. Suspected tumor recurrence in the ipsilateral breast, chest wall structures or lower (level I ± II) axillary nodal areas must be confirmed by biopsy or cytology. Histologic or cytologic confirmation of tumor is recommended for internal mammary or infraclavicular/high axillary nodal recurrence. A distant recurrence is defined as development of tumor in areas other than the local/regional area that is documented by a positive cytology aspirate, biopsy, or imaging studies.

11.23 New primary (NEWP): A new primary is defined as the development of contralateral breast cancer or a second cancer other than squamous or basal cell carcinoma of the skin, carcinoma in situ of the cervix or LCIS of the breast that is histologically confirmed.

11.3 Further treatment after the documentation of a breast cancer recurrence or second primary cancer is left to the discretion of the treating physician.

12.0 Descriptive Factors: None.

13.0 Treatment/Follow–up Decision at Evaluation of Patient

13.1 Patients who have not recurred at time of their reassessment and have <u>not</u> experienced intolerable toxicity may continue protocol treatment at the same dose level for a maximum of 6 cycles or until progression of disease, a second primary or an intolerable adverse event occurs.

13.11 Patients who complete 6 cycles of treatment without disease recurrence, a second primary or intolerable toxicity will go to the observation phase of the study for a maximum of 2 years post randomization. Patients who develop recurrent disease during the observational phase will go to event monitoring phase for a maximum of 2 years post randomization.

13.2 Patients who develop progression of disease, a second primary or intolerable toxicity will be removed from protocol treatment and go to the event monitoring phase of the study. Subsequent treatment is at the discretion of the treating physician.

13.3 Patients may refuse further protocol treatment at any time and go to the event-monitoring phase of the study.

13.4 If a patient is declared ineligible by the study team, on-study material, treatment evaluation forms, an End of Active Treatment/Cancel Notification Form must be submitted. No further follow-up after notification of ineligibility is required.

13.5 If a patient is declared a cancel by the study team before any study treatment is given, on-study material and End of Active Treatment/Cancel Notification Form must be submitted. No further follow-up is required.

13.6 If patient is found on central review to be MUC1 negative, the patient will be considered a cancel. The Pre-Registration Screening Failure Form must be submitted. No further data submission is necessary.

13.7 There will be no replacement of patients who discontinue or are removed from the protocol for any reason.

13.8 A patient is deemed a *cancel* if he/she is removed from study for any reason after pre-registration but prior to registration. The Pre-Registration Screening Failure Form must be submitted. No further data submission is necessary.

14.0 Correlative/Translational Studies

14.1 Description of Assays

Active vaccines for the immunotherapy of solid tumors have met with only limited success. It is our hypothesis that the causes of this failure are multifactorial and can be improved by the inclusion of stringent patient selection criteria, careful dose titration based on immunologic response monitoring, and correlation of immunologically based dosing parameters with clinical outcome. The following sections define the strategies that will be employed in this trial to evaluate immunologic response to MUC1, and HER-2 peptides.

14.11 Immune Responses to T Helper and CTL Epitopes

14.111 Elispot

Estimates of frequencies of peptide-specific, IFNγ- and IL-5-producing cytotoxic T lymphocytes and helper T lymphocytes will be obtained by ELISPOT assays following *in vitro* stimulation with peptide-sensitized stimulator cells [74, 75]. IL-5 production, rather than that of IL-4, will be assayed because of the increased signal:noise ratio [74]. $CD8^+$ and $CD4^+$ T cells will be positively selected by magnetic activated cell sorting (MACS, Miltenyi Biotech) from cryopreserved and thawed peripheral blood lymphocyte buffy coat. Antigen-presenting cells (APCs) will also be isolated from $CD4^-/CD8^-$ cell population by MACS (beads and reagents purchased from Miltenyi Biotech). $CD8^+$ and $CD4^+$ responder T cells will be stimulated with irradiated APCs pulsed with the target peptides used for vaccination. After 5 days of co-culture, the responding cells will be diluted, titrated, and re-stimulated with APCs pulsed with target peptides for 24 hours in 96 well microtiter ELISPOT plates coated with IFNγ- or IL-5-specific capture antibody (ELISPOT Kit purchased from MABTECH, Stockholm, Sweden). The target peptides for re-stimulation include the peptide used for primary stimulation (MUC1 and HER-2 peptides) and a negative control peptide (YIGEVLVSV). The wells are washed and treated with ALP-conjugated secondary antibody and cytokine-producing spots detected using appropriate substrate (all reagents are provided in the kit). After stopping the reaction, the developed microtiter plates are shipped to Zellnet Consulting in New York for evaluation of number of spot-producing cells for each responder cell titration. All analyses are performed by the consulting firm and data provided electronically to the investigator. The difference between the frequency of spot-producing cells obtained with the target peptides and control peptide will determine the frequency of peptide-specific, cytokine-producing $CD4^+$ or $CD8^+$ T cells.

14.112 Tetramers

The estimation of frequencies of CTLs that recognize specific peptides bound to class I molecules became increasingly easier and more quantifiable with the construction and application of class I tetramers [11, 76, 77]. Class I MHC tetramers are composed of a complex of four HLA MHC class I molecules each bound to the specific peptide and conjugated with a fluorescent protein (MHC Tetramer-Streptavidin-Phycoerythrin (SA-PE)). We will use MUC1 M1.1 peptide (STAPPVHNV) and HER-2/neu peptide 9_{435} (ILHNGAYSL). To detect epitope spreading, we will also use HER-2/neu peptide$_{369\text{-}377}$ (KIFGSLAFL). As a negative control, we will use the multi-allele negative tetramer from Beckman Coulter (T01044). For positive control we will use the HLA-A0201 CMV PP65 tetramer (NLVPMVATV) from Beckman Coulter (T01009). Tetramers of HLA-A2 molecules are commercially available (Beckman Coulter). On the day of staining, test PBLs are thawed, washed, and resuspended in the manufacturer's recommended staining buffer (PBS) at 1 x 10^6 cells/ml. Tetramers and any additional antibodies (such as anti-CD8 or anti-CD3 conjugated to a different fluor such as FITC) are added to the cell volumes and incubated for 30 min at room temperature. The cell suspension is then washed with PBS and resuspended in PBS with 0.5% formaldehyde (Fixative Reagent) and analyzed by flow cytometry with FACSCAN instrumentation and CellQuest software (BD Biosciences); a minimum of 5 x 10^5 cells/sample are analyzed for accurate estimation of $CD8^+$ CTLs with low frequencies. The analysis involves (1) gating on lymphocytes using forward and side-scatter; (2) gating on FITC-positive PBLs that stain with anti-CD3 or anti-CD8, and (3) analyzing the gated cells for PE and FITC staining. The frequency of doubly stained cells ($tetramer^+/CD8^+$) will be estimated for each of three replicate tubes for calculation of the mean frequency ($\pm$sd).

14.12 Antigenic Profiling

14.121 Expression of Class I HLA Antigens on tumor tissue.

Initial entry criteria require HLA-A typing of peripheral blood with subsequent confirmation of HLA class I antigen expression on tumor cells by immunohistochemistry. One of the mechanisms by which tumors are postulated to evade the immune response is by down regulation of classical HLA molecules necessary for antigen presentation.

14.122 Tumor Expression of MUC1

Tumor blocks will be used to determine the levels of expression of MUC1 on breast cancer tumor cells obtained at the time of most recent surgical resection. MUC1 expression will be determined by positive staining with one of several antibodies to MUC1 (HMFG-2, BC-2, or B27.29). Negative controls will be incubated with PBS instead of monoclonal antibody. Staining of cytoplasm and plasma membrane will be evaluated. Cells will be considered positive when at least one of these components is stained. Antibody staining patterns will be scored in a semi quantitative manner from +1 to +3.

14.13 Sample Schedule

14.131 Blood

100 mL of blood (about 7 tablespoons) will be collected (heparin) prior to registration, prior to cycles 3, 5 and 7 of therapy as well as every 3 months after conclusion of active therapy until 24 months following registration. Prior to each study blood collection a complete blood count will be performed. If the serum hemoglobin is less than 10.0, the study sample will not be collected. Study sample collection will be postponed for the next study visit.

14.132 Tumor

Tumor blocks will be collected from the patient's most recent surgery prior to study registration. Sections from the tumor blocks will be stained for MUC1. Any/all remaining tissue samples will be returned to the clinical file. Any/all excess samples will be destroyed.

14.14 Sample Preparation

14.141 Blood

Peripheral blood lymphocytes (PBLs) are enriched by flotation over Ficoll-Hypaque and frozen in aliquots in 10% DMSO for storage at -150^{o}C. Percentages of CD4^{+} and CD8^{+} T cells, B cells, monocytes, and dendritic cells are estimated by flow cytometry with a panel of specific monoclonal antibodies. In addition, proliferation assays (3H-thymidine uptake) are performed to estimate T cell responses to polyclonal stimulus (phytohemagglutinin), target antigens (MUC1 and HER-2/neu) and a recall antigen (tetanus toxoid). These two sets of experiments are important for estimating the representation of individual lymphoid populations and evaluating overall T cell responsiveness. CD8^{+} (CTLs) and CD4^{+} (HTLs) are positively purified from cryopreserved and thawed PBLs by magnetic bead separation (Miltenyi Biotek). Additionally, serum will be collected and stored from each of these samples. Cells will then be frozen and stored at -150^{o} for future use.

14.3 Delayed-type hypersensitivity (DTH) skin testing

Skin testing (baseline - prior to registration) will be coordinated at each participating site. A typical panel includes candida, mumps, PPD, and trichophyton. Other antigens may be substituted in the event of antigen unavailability. Patients will return for 1-2 follow-up measurements consistent with site procedures.

15.0 Drug Information

15.1 MUC-1 (STAPPVHNV) - *Investigational supply*

15.11 Other Names: epithelial membrane antigen (EMA), polymorphic epithelial antigen (PEM), DF3 antigen, Ca1, MAM-6, H23

15.12 Formulation and Storage: MUC-1 will be provided as a powder in a glass vial with a Teflon coated stopper. Each vial will contain 1 mg of peptide. The product is frozen at -20^{o}C until use.

15.13 Drug Procurement and Accountability: to be purchased from Clinalfa

15.2 HER-2 Peptide-1 (ILHNGAYSL) - *Investigational supply*

15.21 Other Names: erbB2, neu

15.22 Formulation and Storage: HER-2 Peptide-1 will be provided as a powder in a glass vial with a Teflon coated stopper. Each vial will contain 1 mg of peptide. The product is frozen at -20^{o}C until use.

15.23 Drug Procurement and Accountability: purchased from Clinalfa

15.3 HER-2 Peptide-2 (KVPIKWMALESILRRRF) - *Investigational supply*

15.31 Other Names: erbB2, neu

15.32 Formulation and Storage: HER-2 Peptide 2 will be provided as a powder in a glass vial with a Teflon coated stopper. Each vial will contain 1 mg of peptide. The product is frozen at -20^{o}C until use.

15.33 Drug Procurement and Accountability: purchased from Clinalfa

15.4 Montanide ISA-51 Adjuvant [MONTAN] - *Investigational supply*

15.41 Formulation and Storage

Montanide ISA-51 or Montanide ISA-51 VG is an oil-based adjuvant product similar to Incomplete Freund's Adjuvant. When mixed with a water-based solution at a 1:1 w/w ratio, it forms a water-in-oil emulsion. It consists of highly purified oil, Drakol VR, and a surfactant, mannide oleate. Montanide ISA-51 is manufactured by Seppic, Inc., and is provided in amber glass ampoules or vials containing 3 mL of the solution. Montanide ISA-51 or Montanide ISA-51 VG will be purchased from Seppic Inc.

15.42 Mode of Action

Acts to enhance immune response to vaccination; the precise mode of action is unknown.

15.43 Storage and Stability

The solution is stored at controlled room temperature. Exposure to cold temperatures may result in a clouded solution, which should be discarded. An expiration date is printed on the ampoule label.

15.44 Compatibilities/Incompatibilities

The oil may break down the rubber tip of the plunger on syringes; it is advisable to use a different syringe for each ampoule or vial. Do not allow the Montanide ISA-51 to be in direct contact with the rubber tip of the plunger for more time than is necessary to withdraw the solution and inject it into the peptide vial. Fresh syringes will be needed to withdraw the emulsified vaccine from the vaccine vial. Once the emulsion is made, there is less interaction of the oil directly with the rubber tip of the plunger.

15.45 Drug Procurement and Accountability

Montanide ISA-51 will be purchased from Seppic Inc. using study grant funds.

15.5 GM-CSF (sargramostim, Leukine®)

15.51 Preparation and Storage

Liquid sargramostim (used in this study) is available in vials containing 500 mcg/mL (2.8×10^6 IU/mL) sargramostim. LEUKINE liquid should be refrigerated at 2-8°C (36-46°F). Do not freeze or shake. Do not use beyond the expiration date printed on the vial.

15.52 Known Potential Toxicities

Fever, chills, asthenia, malaise, numbness, increased sensitivity to touch, loss of balance, dizziness, rash, peripheral edema, dyspnea, headache, pericardial effusion, bone pain, arthralgia, nausea, vomiting, loss of appetite, developing or worsening of kidney or liver problems, difficulty breathing, shortness of breath, redness of the skin, facial flushing, rapid or irregular heartbeat or other heart problems, low blood pressure, myalgia, and serious allergic reactions such as a severe asthmas attack.

15.53 Drug Procurement:

Leukine 500 mcg vials are available commercially. Drug will be purchased for this project using study grant funds. Patients will not be charged for the GM-CSF.

15.6 CpG-7909 (PF-3512676, Pfizer Pharmaceuticals, Inc)

15.61 Preparation and Storage: PF-3512676 (Injection) is formulated as a sterile phosphate buffered saline solution suitable for parenteral administration. This sterile and pyrogen-free solution contains no preservatives; vials are intended for single entry to prevent contamination. The drug product is packaged in clear, type I USP glass vials with Teflon-coated stopper closures and flip-caps. The drug product should be stored under refrigeration (2 to 8^{o}C). Each vial provides 15mg/mL (1.2mL fill volume).

15.62 Known potential toxicities: The list of reported serious adverse events with the use of CpG-7909 demonstrates the following toxicities:

15.621 **Related**: reactive follicular lymphatic hyperplasia.

15.622 **Possibly Related**: anemia, superior vena cava syndrome, dyspnea, malignant ascites, post-operative bleeding, hepatic failure, renal failure, post-operative wound infection, GI hemorrhage, prolonged coagulation time, bacteriemia, ureteric obstruction, congestive heart failure, DVT, vomiting, dehydration, vein compression, hydronephrosis, urinary retention, proctalgia, hypercalcemia, pleural effusion, subacute inflammatory demyelinating polyneuropathy, pelvic inflammatory disease, unstable angina, myocardial infarction, atrial fibrillation and grand mal seizures.

15.63 Drug Procurement: will be provided free of charge by Pfizer Pharmaceuticals, Inc.

15.7 Vaccine Preparation Instructions

15.71 General Vaccine Preparation Information

Emulsify the peptide(s)/GM-CSF and/or CpG mixture with Montanide ISA-51. Prepare the vials as directed for each group below. Because neither the peptide solution nor the Montanide ISA-51 contains preservatives or bacteriostatics, the prepared peptide vaccines should be administered as soon as possible.

15.711 Arm A

Remove one vial of Montanide ISA-51 or Montanide ISA-51 VG from the study supply. Remove one vial each of MUC1, HER-2 peptide 1, and HER-2 peptide 2 from the freezer and allow them to reach room temperature. Remove a vial of liquid GM-CSF (500 mcg/mL) from the refrigerator and allow to reach room temperature. Withdraw 0.5 mL (250 mcg) of GM-CSF and add to one of the peptide vials. Add 0.5 mL of Sterile Water for Injection to a second peptide vial and 0.5 mL of Sterile Water for Injection to a the third peptide vial. Swirl the vials to dissolve the powder in each vial. Use a 5-6 mL latex-free or glass syringe to withdraw the entire solution from each vial. Attach a stopcock to the syringe. The stopcock should have the off-lever pointed toward the male connector. Use a second 5-6 mL latex-free or glass syringe to withdraw 1.5 mL from the Montanide vial. Attach this syringe to the stopcock. Inject the peptide solution into the syringe containing the Montanide. Continue injecting the mixture back and forth between the two syringes for approximately 5 minutes. Test the emulsion by adding a drop to the surface of chilled sterile water. If the drop disperses over the surface of the water, continue mixing and re-test. This test may be repeated twice. Load two 3 mL syringes with equal volumes of this emulsion prior to use. The nurse will administer the vaccine mixture to the patient as soon as possible.

Arm B

Remove one vial of Montanide ISA-51 or Montanide ISA-51 VG from the study supply. Remove one vial each of MUC1, HER-2 peptide 1, and HER-2 peptide 2 from the freezer and allow them to reach room temperature. Remove a vial of liquid CpG-7909 (PF-3512676, 15mg/mL) from the refrigerator and allow to reach room temperature. Withdraw 0.14 mL of CpG-7909 (approximately 2.1 mg) and add to one of the peptide vials. Add 0.35 mL sterile water to the same vial. Add 0.5 mL of Sterile Water for Injection to a second peptide vial and 0.5 mL of Sterile Water for Injection to the third peptide vial. Swirl the vials to dissolve the powder in each vial. Use a 5-6 mL latex-free or glass syringe to withdraw the intire solution from easch vial. Attach a stopcock to the syringe. The stopcock should have the off-lever pointed toward the male connector. Use a second 5-6 mL latex-free or glass syringe to withdraw 1.5 mL from the Montanide vial Attach this syringe to the stopcock. Inject the peptide solution into the syringe containing the Montanide. Continue injecting the mixture back and forth between the two syringes for approximately 5 minutes. Test the emulsion by adding a drop to the surface of chilled sterile water. If the drop disperses over the surface of the water, continue mixing and re-test. This test may be repeated twice. Load two 3 mL syringes with equal volumes of this emulsion prior to use. The nurse will administer the vaccine mixture to the patient as soon as possible.

Arm C

Remove one vial of Montanide ISA-51 or Montanide ISA-51 VG from the study supply. Remove one vial each of MUC1, HER-2 peptide 1, and HER-2 peptide 2 from the freezer and allow them to reach room temperature. Remove a vial of liquid GM-CSF (500 mcg/mL) from the refrigerator and allow it to reach room temperature. Remove a vial of liquid CpG-7909 (PF-3512676 15mg/mL) from the refrigerator and allow it to reach room temperature. Withdraw 0.14mL of CpG-7909 (approximately 2.1 mg) and add to one of the peptide vials. Add 0.35 mL sterile water to the same vial. Add 0.4 mL (225 mcg) of GM-CSF to a second peptide vial.

Add 0.5 mL of Sterile Water for Injection to the third peptide vial. Swirl the vials to dissolve the powder in each vial. Use a 5-6 mL latex-free or glass syringe to withdraw the entire solution from each vial. Attach a stopcock to the syringe. The stopcock should have the off-lever pointed toward the male connector. Use a second 5-6 mL latex-free or glass syringe to withdraw 1.5 mL from the Montanide vial Attach this syringe to the stopcock. Inject the peptide solution into the syringe containing the Montanide. Continue injecting the mixture back and forth between the two syringes for approximately 5 minutes. Test the emulsion by adding a drop to the surface of chilled sterile water. If the drop disperses over the surface of the water, continue mixing and re-test. This test may be repeated twice. Load two 3 mL syringes with equal volumes of this emulsion prior to use. The nurse will administer the vaccine mixture to the patient as soon as possible.

15.8 Vaccine Administration Information

15.81 Dose Specifics

Each peptide vaccine will consist of a total volume of approximately 2 - 3 mL, containing the correct dose of the peptide(s), and/or GM-CSF and/or CpG. Be sure to confirm the proper cohort and dose level before preparing the product.

15.82 Administration

Vaccinations will be given subcutaneously on day 1 of each treatment cycle. Due to the large volume, each peptide vaccine is administered in 2 shots in a contiguous location in the vicinity of one of the major nodal basins. This basin must not have been dissected.

15.9 Vaccine Side Effects:

15.91 Because of the low dose of GM-CSF used and the slow release nature of the vaccine emulsion, side effects normally seen with systemic treatment doses of GM-CSF should not play a factor in this vaccination treatment. Expected side effects are related to the peptides and Montanide ISA-51. It is possible that the GM-CSF and CpG-7909 may potentiate the reaction seen at the injection site.

15.92 Dermatology/Skin: Injection site reaction, rare granuloma formation, possible development or worsening of pre-existing vitiligo, rash.

15.93 Hepatic: transient rises in liver transaminases.

15.94 Constitutional: Low-grade fever.

16.0 Statistical Considerations and Methodology

16.1 Study goals:

- **Primary goal**: to determine the safety and immunization efficacy of MUC1 and HER-2/neu peptide vaccines combined with CpG, GM-CSF or both, as immune adjuvants suspended in Montanide ISA-51.

- **Secondary goal**: to describe the impact of immunization on clinical outcomes in patients with MUC1 positive breast cancer.

16.2 The study design chosen for this proposal is a stratified randomized design. Toxicities will be carefully monitored and accrual will be suspended if 2 or more of the first six patients experience a grade 4 hematologic toxicity lasting for 5 or more days. In the event of at least two patients experiencing immunologic toxicity ≥ grade 2 or any toxicity ≥ grade 3 accrual will be temporarily suspended for the given treatment arm.

16.3 Accrual: Fifteen eligible patients with MUC1/HER-2 positive breast cancer and no evidence of disease will be randomized to each of the 3 treatment schedules. We anticipate 20% of the patients who pre-register to this study will be found not to have MUC-1 positive disease and 5% of the patients who do have MUC-1 positive disease will either cancel participation prior to starting treatment or will be found to be ineligible. As such, we anticipate preregistering 58 patient to obtain 45 eligible patients who will sign a consent form and start study treatment.

Patients will be assigned to treatment using a dynamic allocation procedure that balances the marginal distribution of type of dominant disease between treatments. The expected accrual rate for this study is approximately 15-20 patients per year at Mayo Clinic Rochester, 5-7 patients per year at Mayo Clinic Arizona and 5-7 patients per year at Mayo Clinic Florida. Enrollment is expected to extend approximately 2.5 years.

16.4 Study Endpoints:

16.41 Primary Endpoints

16.411 The immunologic parameters of interest are: (1) the percentage of CD4+ T cells, CD8+ T cells, B cells, monocytes, and dendritic cells in a patient's peripheral blood sample as estimated by flow cytometry with a panel of monoclonal antibodies and (2) the frequency of both peptide-specific IFN-gamma producing T cells and peptide-specific IL-5 producing T cells estimated by ELISPOT assays following *in vitro* stimulation with peptide-sensitized stimulator cells for the MUC1 and HER-2 peptides.

16.412 The number and severity of hematologic and non-hematologic toxicities reported using the NCI-CTC version 3.0 criteria

16.42 Secondary Endpoints

16.421 Disease-free survival is defined as the time from registration to the documentation of a first failure where a failure is the recurrence (REC) of breast cancer or a diagnosis of a second primary cancer (NEWP).

16.422 Overall survival is defined as the time from registration to death due to any cause.

16.43 Immunologic Parameters

16.431 All eligible patients who have completed one cycle of treatment are evaluable for the analysis of the immunologic parameters.

16.432 For each of the immunologic parameters, a plot of the parameter level against time will be constructed such that each patient is represented by a line connecting that patient's data points. These plots will enable visual assessment of patterns of change and variability within a parameter as well as a visual assessment of whether the immunologic parameters peak or fall at similar time points.

16.433 Also, for each of the immunologic parameters, a plot of the percent change from pre-treatment levels against time will be constructed such that each patient is represented by a line connecting that patient's data points. These plots will enable visual assessment of time trends within a parameter controlling for pretreatment levels.

16.44 Adverse Events

16.441 All eligible patients who received at least one vaccination are evaluable for toxicity.

16.442 The frequency of those hematologic and non-hematologic toxicities considered at least possibly related to treatment will be tabulated by severity.

16.443 The circumstances surrounding any treatment-related death will be reported.

16.444 As this is a pilot study, no formal hypothesis tests comparing treatment schedules are planned. An immunization strategy will be considered for further testing if at least 70% patients treated with that strategy had a ≥2-fold increase in the percentage of vaccine-peptide specific CD8+ T cells during the course of treatment, with tolerable toxicity.

16.445 The principal investigator and study statistician will review the study every 3 months to identify potential accrual, toxicity, or endpoint problems. In addition, this study will be monitored by the Cancer Center Data Safety Monitoring Board. All patient related clinical data will be entered and maintained online, with reports generated as needed to comply with reporting guidelines.

16.446 It should be noted that representatives of the U.S. Army Medical Research and Merial Command are eligible to review research records as a part of their responsibility to protect human subjects in research.

16.447 If the protocol requires any modifications, deviations or termination prior to completion, all administrative activities will comply with the Protocol Review and Monitoring System of the Mayo Clinic Comprehensive Cancer Center. In addition, all local IRB communications, including deviations from protocol, will be forwarded to the Department of Defense HSRRB, upon local approval.

16.45 Inclusion of Minorities

This study will be available to all eligible patients, regardless of race or ethnic group. There is no information currently available regarding differential agent effects in subjects defined by gender, race, or ethnicity. The planned analyses will, as always, look for differences in treatment effect based on racial groupings. The sample sizes of this pilot study, however, are not sufficient to provide power for such subset analyses.

To predict the characteristics of patients likely to enroll in this trial we have reviewed registration to (non-North American Breast Cancer Intergroup) NCCTG breast cancer clinical trials by race. This revealed that roughly 3% of patients registered into cancer trials during the past five years could be classified as minorities, which would suggest that only 1 or 2 patients in the study sample are expected to be classified as minorities. This small sample precludes the possibility of a separate subset analysis beyond simple inspection of results for the 1 or 2 minority patients.

17.0 Pathology Considerations for Pre-registration Central Pathology Review

17.1 There will be a central pre-registration review of MUC-1 expression.

17.11 The following materials are to be submitted.

- Central Testing of MUC1 Expression Form
- Surgical Pathology and Operative Report
- One H&E and 6 unstained slides

Slides should be placed in appropriate slide container and labeled with the protocol number, study patient number, and patient initials. Slides will be stained in Dr. Sandra Gendler's lab. All samples should be submitted to:

Cathy S. Madsen
Senior Research Technologist for Dr. Sandra Gendler
Mayo Clinic Scottsdale
Johnson Research Building SCJ 2-221
13400 E. Shea Blvd.
Scottsdale AZ 85259

Notify Cathy S. Madsen by phone (79)2-6064, or e-mail (cathy.madsen@mayo.edu) that samples have been shipped.

The slides will be logged and forwarded to Dr. Ann McCullough in Scottsdale for central pre-registration review of MUC1 expression.

Slides and correlating paperwork will be returned to Cathy Madsen for Dr. Gendler's lab.

1 8.0 Records and Data Entry Procedures

18.1 Data Entry Timetable

Forms	Active-Monitoring Phase (Compliance with Test Schedule)				Event-Monitoring Phase [1] (Completion of Active-Monitoring Phase)				At Each Occurrence			
		Initial material	Follow-up material									
	Pre-Reg	≤2 weeks after registration	At each evaluation	At end of treatment	q.3 months until PD	At PD	After PD q.3 mos.	Death	ADR/ AER	New Primary	Grade 4 or 5 Non-AER Reportable Events/ Hospitalization	Late Adverse Event
On-Study Form		X										
Blood Specimen Submission Form [2]		X	X									
Pathology Materials (see Section 17.0)	X											
Pre-reg Screening Failure Form [8]	X											
Baseline Adverse Events Form		X										
Measurement Form		X	X	X								
Evaluation/Treatment Form			X[7]	X								
Evaluation/Observation Form			X[5]									
DTH Laboratory Form [3]		X	X									
Interval Laboratory Form [4]		X	X	X								
Adverse Event Form			X	X								
End of Active Treatment/ Cancel Notification Form		X[6]		X								
Event Monitoring Form				X	X	X	X	X		X		X
Concurrent Treatment Form		X	X	X								
ADR/AER (See Section 10)									X			
Secondary AML/MDS Report Form (See Section 10)									X			
Grade 4 or 5 Non-AER Reportable Events/Hospitalization Form (See Section 10.0)											X	

1. If a patient is still alive 2 years after registration, no further follow-up is required.
2. Research blood samples will be performed prior to registration, prior to cycles 3, 5, and 7 of therapy as well as every 3 months after conclusion of active therapy until 24 months following registration.
3. At baseline and prior to cycle 6 only.
4. At baseline, prior to each subsequent treatment and at 4 weeks after last treatment.
5. Complete at each evaluation during Observation (see Section 4.0).
6. Submit if withdrawal/refusal prior to beginning protocol therapy occurs.
7. Complete at each evaluation during Active Treatment (see Section 4.0).
8. Complete only if patient is NOT registered after he/she is pre-registered

19.0 Budget Considerations

19.1 Costs charged to patient: routine clinical care.

19.2 Tests and procedures to be research funded: HLA typing, tumor typing. DTH testing and serum pregnancy tests. Funding will be provided by the Department of Defense (DOD).

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Appendix I

ECOG PERFORMANCE STATUS

Grade

Grade	
0	Fully active, able to carry on all pre-disease activities without restriction (Karnofsky 90-100).
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work (Karnofsky 70-80).
2	Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50 percent of waking hours (Karnofsky 50-60).
3	Capable of only limited self-care, confined to bed or chair 50 percent or more of waking hours (Karnofsky 30-40).
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair (Karnofsky 10-20).
5	Dead

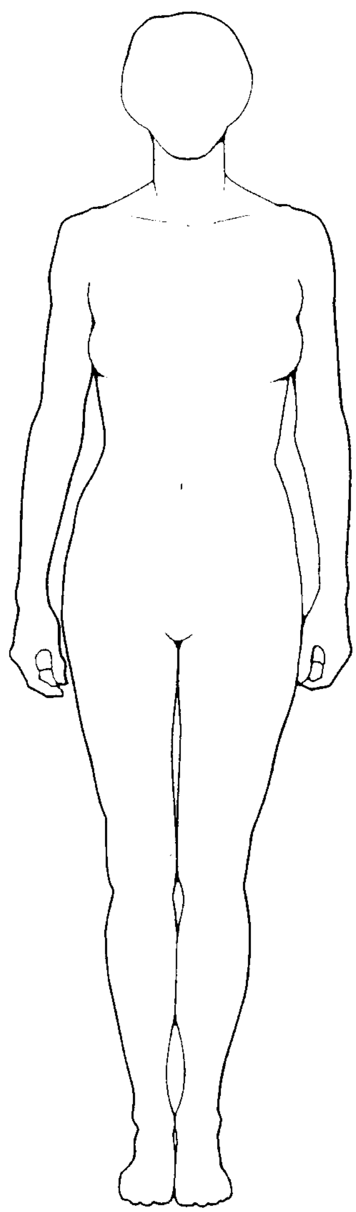
Appendix II

Site Injection Record

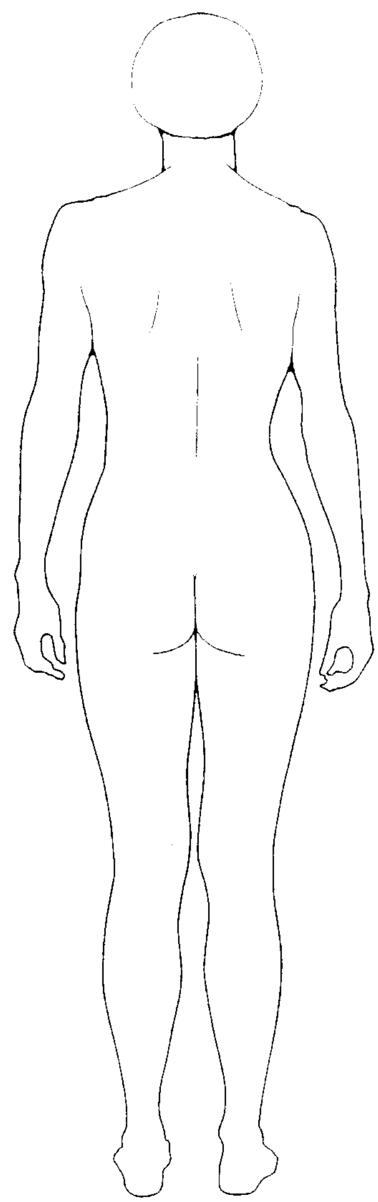
Protocol #: ________________ Patient #: _________________ Patient Initials: ___ ___ ___
F M L

Please indicate on the diagrams below all sites of vaccine injection (circle).

Date of determination: __ __ / __ __ / __ __
M D Y



Anterior



Posterior

Consent Form for Participation in a Research Study

TITLE: MC0338, "MUC1/HER-2/neu Peptide Based Immunotherapeutic Vaccines for Breast Adenocarcinomas"

IRB #: 782-05 00

RESEARCHER: Dr. S. N. Markovic and colleagues.

PROTOCOL LAST APPROVED BY INSTITUTIONAL REVIEW BOARD: September 23, 2009

THIS FORM APPROVED: September 23, 2009

This is an important form. Please read it carefully. It tells you what you need to know about this research study. If you agree to take part in this study, you need to sign this form. Your signature means that you have been told about the study and what the risks are. Your signature on this form also means that you want to take part in this study.

Why is this research study being done?

We are inviting you to participate in this study because of your history of resected breast cancer for which you have already received standard therapy and have no evidence of relapse. Your good overall health and no other history of cancer make it possible for us to offer you participation on this clinical study.

This study is being done to evaluate three different preparations of a breast cancer vaccine to stimulate anti-cancer (T-cell) immune responses and any side effects associated with these vaccinations. The breast cancer vaccine (MUC-1/HER-2/neu peptides) will be combined with one of two immune boosting agents (CpG or GM-CSF) or with immune boosting agents together. These immune boosting agents are believed to be able to make the vaccine more effective. Whether or not this will protect you from breast cancer is still unknown.

The breast cancer vaccine and one of the immune boosting agents (CpG) have not been approved by the Food and Drug Administration (FDA) for commercial use; however, FDA has permitted their use in this research study. GM-CSF is commercially available for use in clinical practice. Laboratory experiments have shown that both of these immune boosting agents are able to make the vaccines more effective in generating an immune response. In laboratory animals, both agents are very effective in boosting anti-tumor immune responses. However, in clinical trials, neither GM-CSF nor CpG have shown effects against cancer, but they both seem to improve the effectiveness of some vaccines. One of the goals of this study is to determine if the unique application of GM-CSF and/or CpG in this study, based on our laboratory data, will make them more likely to improve vaccine immunity.

This study is sponsored by the Department of Defense.

How many people will take part in this research study?

The plan is to have up to 45 people take part in this study at Mayo Clinic Rochester, Florida, and Arizona. Up to 200 people may be screened to find enough eligible people to begin the study.

What will happen in this research study?

Before you enter the study, you will have a physical examination and blood tests to make sure that you qualify to take part in this study. About 6 tablespoons of blood will be taken for testing.

If you qualify to take part, you will have blood taken for immunologic testing (to see how your immune system is working) for the study as well as a skin test (allergy test). You will then be randomly assigned (as in the toss of the dice) to be treated with one of three breast cancer vaccines combinations.

Each vaccination will consist of one or two subcutaneous (under the skin) injections of about one half teaspoonful (2ml) of the cancer vaccine and boosting agent combination. The vaccine will be given with Montanide ISA 51, which is an oil that is mixed with the vaccine so that the vaccine can be released into the body slowly. The vaccine will be injected under the skin in areas where there has been no surgery. Usual areas of vaccination include the skin of the upper arms and legs. Vaccinations will be repeated every 4 weeks for 6 months. Before every vaccination, you will be seen by your doctor, examined and 6 tablespoons of blood will be collected to study the development of the anti-cancer immune response. Skin tests will be done prior to vaccinations #6 (see the following table). Evaluations for the cancer (body scans) will be done if and when your doctor thinks it is necessary.

Pre-Study	• Existing tissue samples from your most recent surgery before study registration will be reviewed to determine if you qualify for the study, based on the MUC1 characteristic in the sample. • Routine[1] and research blood tests • Cancer evaluation (scans) • Physical examination • Skin test
Vaccination #1 (month 1)	• Vaccine treatment
Vaccination #2 (month 2)	• Routine blood test collection • Physical examination • Vaccine treatment
Vaccination #3 (month 3)	• Routine and research blood test collection • Physical examination • Cancer evaluation (scans) • Vaccine treatment
Vaccination #4 (month 4)	• Routine blood test collection • Physical examination • Vaccine treatment
Vaccination #5 (month 5)	• Routine and research blood test collection • Physical examination • Vaccine treatment
Vaccination #6 (month 6)	• Routine and research blood test collection • Physical examination • Cancer evaluation (scans) • Vaccine treatment • Skin test
Every 3 months after the first six months until 2 years after first vaccination	• Physical examination • Routine blood tests • Cancer evaluation (scans, at the discretion of the physician)
Every 3 months after the first six months until 2 years after first vaccination	• Research bloods

1. Routine blood tests include: complete blood count and blood chemistries.

How long will I be in this research study?

You will be on the study for 6 months, and you will be seen in follow-up until -2 years after your first vaccination.

Are there reasons I might leave this research study early?

Taking part in this research study is your decision. You may decide to stop at any time. You should tell the study doctor if you decide to stop and you will be advised whether any additional tests may need to be done for your safety.

In addition, the investigators or Mayo may stop you from taking part in this study at any time if it is in your best interest, if you do not follow the study rules, or if the study is stopped.

Will any biological sample(s) be stored and used for research in the future?

No. Your samples will be used as described for this study, and then will be destroyed.

What are the risks of this research study?

You will be closely watched by the study team for any side effects. If side effects happen, the study team will take the necessary steps to treat them. This may include stopping the medication and/or stopping the study.

The possible side effects of the following: MUC-1/HER-2/neu, breast cancer vaccine, Montanide ISA 51 (the oil), CpG (immune booster), and GM-CSF (immune booster) include:

Common:

- Injection site reactions: discomfort, rash, redness, firmness, warmth, bleeding, tenderness to touch, numbness, tingling, itching.
- Systemic reactions: skin rash, itching, sweating, muscle aches, joint aches, fatigue.

Rare:

- Injection site reactions: pain, ulceration.
- Systemic reactions: low blood counts, difficulty breathing, heart problems, blood clots, seizures, infection, kidney and liver problems, headache, stomach pain, cough.

Although many of these reactions are similar to vaccines that you may have received in the past, the listed side effects that could happen might be more severe. However, we expect that all of these reactions will be very mild. Treatment for these reactions will depend on the type and severity of the reaction. Treatments will be directed at suppressing the immune reaction to the vaccine and may range from mild anti-allergic/anti-inflammatory treatments (for example topical hydrocortisone or Motrin) to more powerful anti-inflammatory therapy including corticosteroids. If severe reactions happen, they may require hospitalization and possibly even minor surgery (severe local skin reactions).

When GM-CSF has been given at higher doses as a daily injection the following side effects have also been reported: diarrhea, general weakness, fever, chills, nausea, vomiting, loss of appetite, headache, pain in the bones, joints and muscles. Most of the symptoms were mild or moderate in severity and were less after taking acetaminophen (Tylenol). Other side effects which happened very rarely were: difficulty breathing, rapid or irregular heart beat or other heart problems, swelling. Even less common, reported side effects have been the following: 1) increased white cells in the lungs with breathing problems; 2) a syndrome of shortness of breath, low oxygen in the blood, redness in the skin, low blood pressure and dizziness when you stand up or a loss of balance and partial loss of consciousness; 3) serious allergic reactions (like a very severe asthma attack); 4) blood clotting; 5) facial flushing; 6) kidney or liver problems; 7) worsening of fluid accumulation in the arms, legs, lungs or around the heart which may cause problems with breathing or heart failure; 8) patients with heart, lung, kidney or liver problems may have worsening of their symptoms following GM-CSF; and 9) nerve toxicity (weakness, shooting pains, numbness, increased sensitivity to touch, loss of balance, dizziness)

Skin testing: The risks and discomfort of skin testing are minimal and usually limited to bleeding, bruising, or infection at the injection site.

Blood draws: Drawing blood may cause slight pain and a small risk of bleeding, bruising, or infection at the injection site.

There is not enough medical information to know what the risks might be to a breast-fed infant or to an unborn child carried by a woman who takes part in this study. Therefore all women who can become pregnant and are sexually active, or their sexual partners, must use birth control measures while in this study. The following birth control measures are acceptable: diaphragm, birth control pills, injections, intrauterine device (IUD), surgical sterilization, under the skin implants, abstinence. Breast-feeding mothers must stop breast-feeding to take part in this study. Women who can become pregnant must have a pregnancy test before taking part in this study. For the pregnancy test, you will give a blood sample taken from a vein in your arm with a needle within 7days before you enter the study. You will be told the results of the pregnancy test. If the pregnancy test is positive, you will not be able to take part in the study.

While you are taking part in this study, you are at risk for the following side effects. You should talk to your study doctor and/or your medical doctor about these side effects. There also may be other side effects that are not known. Side effects may range from mild to life-threatening. Other drugs may be given to lessen side effects. Many side effects go away shortly after the vaccine treatments are stopped, but in some cases side effects can be serious, long lasting, or may never go away. There may be a risk of death.

Are there benefits to taking part in this research study?

This study may not make your health better. However, the information learned may benefit future patients with breast cancer.

What other choices do I have if I don't take part in this research study?

You do not have to be in this study to receive care for your condition. Your other choices may include participation on other clinical studies to no other care at all. You will have regular appointments with your doctor who will check your condition. You should talk to your doctor about your choices before you decide if you will take part in this study.

Will I need to pay for the tests and procedures?

You will not need to pay for any tests and exams that are done just for this research study, including the research blood tests, skin tests and office visits done only for this research study. You will not need to pay for the vaccine used in this study. However, you and/or your health plan will need to pay for all other tests and procedures that are part of this study because they are needed for your regular medical care. You or your health plan might have to pay for other drugs or treatment given to help control side effects. Before you take part in this study, you should call your health insurer to find out if the cost of these tests, procedures, and/or the device will be paid for by the plan. Some health insurers will not pay for these costs. You will have to pay for any costs not covered by your health insurer. If you have questions while at the Clinic, please go to the Admissions and Business Services office, or you may call Patient Account Services at (507) 287-1819.

What happens if I am injured because I took part in this research study?

If you have side effects from the study treatment, you need to report them to the researcher and your regular physician, and you will be treated as needed. Mayo will bill you or your insurer for these services at the usual charge. Mayo will not offer free medical care or payment for any bad side effects from taking part in this study.

If you are hurt or get sick because of this research study, you can receive medical care at an Army hospital or clinic free of charge. You will only be treated for injuries that are directly caused by the research study. The Army will not pay for your transportation to and from the hospital or clinic. If you have questions about this medical care, talk to the principal investigator for this study, Svetomir N. Markovic, M.D., Ph.D. If you pay out-of-pocket for medical care elsewhere for injuries caused by this research study, contact the principal investigator. If the issue cannot be resolved, contact the U.S. Army Medical Research and Materiel Command (USAMRMC) Office of the Staff Judge Advocate (legal office) at (301) 619-7663/2221.

What are my rights if I take part in this research study?

Taking part in this research study does not take away any other rights or benefits you might have if you did not take part in the study. Taking part in this study does not give you any special privileges. You will not be penalized in any way if you decide not to take part or if you stop after you start the study. Specifically, you do not have to be in this study to receive or continue to receive medical care from Mayo Clinic. If you stop the study you would still receive medical care for your condition although you might not be able to get the study drug.

You will be told of important new findings or any changes in the study or procedures that may affect you or your willingness to continue in the study.

Who can answer my questions?

You may talk to Dr. Svetomir N. Markovic at any time about any question you have on this study. You may contact Dr. Markovic (or an associate) by calling the Mayo operator at telephone (507) 284-2511.

You can get more information about Mayo policies, the conduct of this study, or the rights of research participants from Marcia Andresen-Reid, the administrator of the Mayo Clinic Office for Human Research Protection, telephone (507) 266-4000 or toll free (866) 273-4681.

Where can I get more information about clinical trials?

You may call the NCI's Cancer Information Service at 1-800-4-CANCER (1-800-422-6237) or TTY: 1-800-332-8615

Visit the NCI's Web sites: Cancer Trials: comprehensive clinical trials information
http://cancertrials.nci.nih.gov

CancerNet^{TM}: accurate cancer information including PDQ
http://cancernet.nci.nih.gov

Authorization To Use And Disclose Protected Health Information

Your privacy is important to us, and we want to protect it as much as possible. By signing this form, you authorize Mayo Clinic Rochester and the investigators to use and disclose any information created or collected in the course of your participation in this research protocol. This information might be in different places, including your original medical record, but we will only disclose information that is related to this research protocol for the purposes listed below.

This information will be given out for the proper monitoring of the study, checking the accuracy of study data, analyzing the study data, and other purposes necessary for the proper conduct and reporting of this study. If some of the information is reported in published medical journals or scientific discussions, it will be done in a way that does not directly identify you.

The study data sent by the study doctor to the sponsor does not include your name, address, social security number, or other information that directly identifies you. Instead, the study doctor assigns a code number to the study data and may use your initials. Some study data sent to the sponsor may contain information that could be used (perhaps in combination with other information) to identify you (e.g., date of birth). If you have questions about the specific health information that will be sent to the sponsor, you should ask the study doctor.

This information may be given to other researchers in this study (including those at other institutions), representatives of the sponsor of the study, U. S. Army Medical Research and Material Command, or private, state or federal government parties or regulatory authorities (U.S. and other countries) responsible for overseeing this research. These may include the Food and Drug Administration, the Office for Human Research Protections, or other offices within the Department of Health and Human Services, and the Mayo Clinic Office for Human Research Protections or other Mayo groups involved in protecting research subjects.

If this information is given out to anyone outside of Mayo, the information may no longer be protected by federal privacy regulations and may be given out by the person or entity that receives the information. However, Mayo will take steps to help other parties understand the need to keep this information confidential.

This authorization lasts until the end of the study. The study does not end until all data have been collected, checked (or audited) and analyzed. Sometimes this can be years after your study visits have ended. For example, this could happen if the results of the study are filed with a regulatory agency like the Food and Drug Administration.

You may stop this authorization at any time by writing to the following address:

Mayo Clinic
Office for Human Research Protection
ATTN: Notice of Revocation of Authorization
200 1st Street SW
Rochester, MN 55905

If you stop authorization, Mayo may continue to use your information already collected as part of this study, but will not collect any new information.

If you do not sign this authorization, or later stop authorization, you may not be able to receive study treatment.

What Other Things Might the Sponsor do with Study Data?

In addition to the uses listed above, companies that sponsor studies often use study data for other purposes that are not part of the study. For example, the company might use the study data for research purposes to support the scientific objectives of the study described in this consent document, to learn more about the effects (good and bad) of any drug, device or treatment included in the study, to better understand the disease(s) included in the study, or to improve the design of future studies. Also, the company might share the study data with other companies it does business with. The company might do these things during the study, or after the study has ended, and would not have to ask for your permission to do so. The sponsor might still use study data, even after you stop your authorization, or the authorization expires, as long as the study data was collected before your authorization stopped or expired. The ways in which the study data could be used in the future may not be known now, so we can't give you the details.

A copy of this form will be placed in your medical record.

I have had an opportunity to have my questions answered. I have been given a copy of this form. I agree to take part in this research study.

____________	______________________________	____________
(Date / Time)	(Printed Name of Participant)	(Clinic Number)
	______________________________	
	(Signature of Participant)	
____________	______________________________	
(Date / Time)	(Printed Name of Individual Obtaining Consent)	
	______________________________	
	(Signature of Individual Obtaining Consent)	

STATEMENT OF WORK

Task 1 - To assess the effectiveness of vaccine formulations against MUC1 in the prevention and treatment of spontaneous breast carcinomas in mice

a. First immunization strategy - BLP2S (MUC1 peptide-liposomes, IL-2) Thirty-five MMT mice have been randomized onto the study, which is already in progress. Additional mice will be enrolled until the target number of 18 mice per arm is obtained. Enrollment should be completed by December and the study should be closed by the end of May 2001. Tumors develop over a 24 week period, at which time tumor weight is approximately 10% of the animal weight and the study is closed.

b. Dendritic cell/tumor cell fusion strategy - Forty MMT animals have been randomized onto this study, which began May *IS,* 2000. Additional mice will be enrolled until the target number of 18 mice per arm is obtained. The study will close by the end of May 2001.

c. The adenoassociated virus vector for expressing full-length MUCI in dendritic cells is under construction. It should be completed within the first six months from the start of the grant. Animals will be enrolled at that time and the immunizations will be performed during the second half of the first year of the grant and in the beginning of the second year of the grant.

d. Dendritic cells pulsed with MUCI peptide and the Montanide-ISAS1 vaccine will begin during year one and extend into year two of the grant.

For all of these studies, it takes about one year to complete enrollment of the mice and the immunizations. Tumors develop by about week 6 or 7 and reach 10% of body weight by about 24 weeks. All of the animal studies will be completed by the end of year two.

Determinations of immune response against MUC1 will occur throughout the first two years. Animals are bled monthly and assays performed throughout. Pathological examination of tissues, assessment of metastasis and autoimmunity can occur only when mice are sacrificed.

A sample size of 18 mice per arm of the immunization schedule yields a power of 80% for detecting a 10% change in the rate of tumor growth using a two sample t-test with a two-sided significant level of 0.05. Ninety MMT animals will be randomized to each of the immunization schedules (5 arms in each) in each trial. For the DC/tumor fusion vaccine, additional tumor-bearing mice must be generated to supply the tumor cells for fusion. From three MMT mice, we obtain about 5×10^7 tumor cells, which are fused with the same number of DCs and irradiated. Three mice produce sufficient number of cells for immunization of 6 study animals (1×10^7 cells per mouse). Immunizations occur every three weeks for nine weeks and subsequent booster immunizations will be performed if there is no progressive disease.
Total mice to be entered on study is 90 x 5 = 450 mice. Breeding involves many more mice, as the yield of each transgene is 50% (½ x ½ = ¼) and there is a 50% chance of obtaining females (1/4 x ½ = 1/8). Thus, the yield of useful animals is 12.5%. A 20% excess of anticipated animals will be bred to account for breeding variability. A 20% excess of animals provides a statistical confidence (lower limit of binomial probability) of at least 90% for all experimental groups. An excel chart detailing animal numbers is included with the budget justification.

Task 2 -Accrual: Patients will be recruited by dedicated study coordinators at Rochester, Scottsdale and Jacksonville Mayo Clinics. A large untapped resource of breast cancer patients exists within the Mayo Health System. Considering all three sites, more than 2000 new breast cancer patients were seen. Most of the patients were stages I, II and III. The total number of women to be accrued for this study is 45. The racial distribution is primarily Caucasian, but cases of other minority groups will be sought. Powered statistics are not available for Phase I trials as the focus is mainly to determine toxicity and ISD. Patients with diagnosed resected stage I breast cancer with “high-risk” features including any of the following (triple negative or HER2 over-expression or amplification), stage II, and stage III are eligible for enrollment if they have no evidence of disease and are HLA-A2 positive and the tumors express MUC1 protein. Patients will be from 3 months to 3 years post end of adjuvant therapy (including Herceptin), although they can be on anti-hormone therapy.

Immunological assays will be performed throughout the last two years of this proposal, as patients are entered on the study and during the immunizations.